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(54) Title: THE USE OF ALPHA-1C-SELECTIVE ADRENOCEPTOR AGONISTS FOR THE TREATMENT OF URINARY INCONTINENCE

(57) Abstract

The present invention provides a method of treating urinary incontinence in a subject which comprises administering to the subject a therapeutically effective amount of an α_{1C} selective agonist which activates a human α_{1C} adrenoceptor at least ten-fold more than it activates a human α_{1A} adrenoceptor and a human α_{1B} adrenoceptor. The present invention further provides a method of inducing contraction of urethra and bladder neck tissues which comprises contacting the urethra and bladder neck tissues with an effective contraction-inducing amount of an α_{1C} selective agonist which activates a human α_{1C} adrenoceptor at least ten-fold more than it activates a human α_{1A} adrenoceptor and a human α_{1B} adrenoceptor. In addition, the invention includes compounds for the treatment of urinary incontinence and for use in inducing contraction of urethra and bladder neck tissues.

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THE USE OF ALPHA-1C-SELECTIVE ADRENOCEPTOR AGONISTS FOR THE TREATMENT OF URINARY INCONTINENCE

Throughout this application, various references are referred to within parentheses. Disclosures of these publications in their entireties are hereby incorporated by reference into this application to more fully describe the state of the art to which this invention pertains. Full bibliographic citations for these references may be found immediately preceding the claims.

Background of the Invention

The designation "α_{1A}" is the appellation recently approved by the IUPHAR Nomenclature Committee for the previously designated "α_{1C}" cloned subtype as outlined in the 1995 Receptor and Ion Channel Nomenclature Supplement (Watson and Girdlestone, 1995). However, the designation α_{1C} is used throughout this application and the supporting tables and figures to refer to the receptor subtype recently renamed "α_{1A}". Since in both the old and new nomenclature there has only been one unique receptor subtype which has been designated α_{1C} (i.e., there is no α_{1C} under the current nomenclature), "α_{1C}" is an unambiguous description of this unique receptor subtype.

Incontinence is a condition characterized by the involuntary loss of urine. It can be divided generally into two types, the first involving an unstable bladder as the underlying cause, and the second involving an insufficiency in bladder outlet closing pressure despite the presence of a stable bladder. The condition may arise from a variety of different pathological, anatomical or neurological factors (Lundberg, 1989).

While the prevalence in females is two fold higher, it also affects males (Lundberg, 1989). The greatest incidence is seen in postmenopausal women. It is estimated that at least 10 million Americans suffer from urinary incontinence (Sand et al., 1990). Incontinence can be treated by surgical and nonsurgical methods. Conservative approaches include physiotherapy (Kegel exercises) and functional electrical stimulation which aim to strengthen the peri-urethral musculature (Walters et al., 1992). Periurethral injection of polytetraflurorethylene is a more invasive procedure intended to augment the urethral support (Sand et al, 1990). The most radical treatment for stress incontinence is surgery, involving a variety of techniques which seek to improve the alignment of the bladder, urethra, and surrounding structures.

A variety of pharmaceutical agents have been employed with varying success to treat urinary incontinence. Drugs useful in reducing the contractility of the bladder include anticholinergics, β-blockers, calcium channel blockers, and tricyclic antidepressants. Estrogen has been used with some success in increasing bladder outlet resistance, particularly in postmenopausal women. Its actions have been attributed to a "mucosal seal effect" resulting from urethral mucosal cell proliferation (Wein, 1987), although there is now some suggestion that it may also contribute to a restoration of α-adrenoceptor expression in the urethra (Wein, 1987).

The most commonly employed agents for increasing bladder outlet resistance are the α-adrenoceptor agonists. These activate α-adrenoceptors located on the smooth muscle cells of the proximal urethra and bladder neck

(Sourander, 1990; Wein, 1987), resulting in contraction and increased closing pressure. The compounds currently employed for this therapy include the non-selective adrenoceptor agents phenylpropanolamine, ephedrine, and phenylephrine (Wein, 1987; Lundberg, 1989). The actions of these drugs are attributable, in part, to direct activation of adrenoceptors and in part to their ability to displace endogenous norepinephrine from sympathetic neurons following uptake into the nerve terminal, a so-called indirect sympathomimetic action (Andersson and Sjögren, 1982). Their lack of selectivity (see Table 3 hereinafter) among the adrenoceptor subtypes and the indirect action of these compounds results in their activating α_1-, α_2-, and β-adrenoceptors in the CNS and in the periphery. As a result, any desired therapeutic effect of these agents may be accompanied by a constellation of undesirable side effects. One major side effect of their use in incontinence is an increase in blood pressure. This effect is dose-dependent and limits the ability to achieve therapeutically effective circulating concentrations of the drug (Andersson and Sjögren, 1982). In addition, these compounds in some patients produce insomnia, anxiety and dizziness as a result of their stimulant actions in the CNS (Andersson and Sjögren, 1982, Wein, 1987).

Another compound which has been evaluated in urinary incontinence is midodrine, a prodrug which is converted *in vivo* to the active phenylethylamine ST-1059. The clinical efficacy of midodrine has not been demonstrated conclusively (Andersson and Sjögren, 1982). Like the above compounds, its effects may be limited by cross-reactivity with other adrenoceptors (see Table 3) which may limit the maximum achievable dose. A better understanding of the subtypes of α-adrenoceptors and

their involvement in various physiological processes will facilitate the development of more efficacious drugs for the treatment of incontinence.

The α-adrenoceptors are specific neuroreceptor proteins located in the peripheral and central nervous systems and on tissues throughout the body. The receptors are important switches for controlling many physiological functions and, thus, represent important targets for drug development. Drugs which interact at these receptors comprise two main classes: agonists, which mimic the endogenous ligands (norepinephrine and epinephrine) in their ability to activate the receptor; and antagonists, which serve to block the actions of the endogenous ligands. Many α-adrenoceptor drugs of both classes have been developed over the past 40 years. Examples in addition to those indicated above, which owe at least part of their action to stimulation of alpha adrenoceptors, include clonidine (agonist; treatment of hypertension), prazosin (antagonist; hypertension), oxymetazoline (agonist, nasal decongestion), and methoxamine (treatment of episodes supraventricular tachycardia). While many of these drugs are effective, they also produce undesirable side effects at therapeutic doses (e.g., clonidine produces dry mouth, sedation and orthostatic hypotension in addition to its antihypertensive effect).

During the past 15 years a more precise understanding of α-adrenoceptors and drugs targeting α-adrenoceptors has emerged. Prior to 1977, only one α-adrenoceptor was known to exist. Between 1977 and 1988, it was accepted by the scientific community that at least two α-adrenoceptors, α_1 and α_2, existed in the central and peripheral nervous systems. Since 1988, new techniques

in molecular biology have led to the identification of at least six distinct α-adrenoceptor proteins which are distributed throughout the central and peripheral nervous systems: α_{1A}, α_{1B}, α_{1C}, α_{2A}, α_{2B} and α_{2C} (Bylund, 1992). In addition to the cloned α-adrenoceptors, several putative α_1 adrenoceptor subtypes have been recently described based upon functional studies in a variety of mammalian tissues. These receptors, which have not been cloned, are described as α_{1H}, α_{1L} and α_{1N} (Murmamatsu, 1995) or "atypical α_1" (Abel, 1995) adrenoceptors. The precise role of each of the subtypes in various physiological responses is only beginning to be understood, but it is clear that distinct subtypes do mediate distinct physiological responses to agonists and antagonists. For example, it has been shown that norepinephrine-induced contractions of the human prostate are mediated by the α_{1C}-adrenoceptor (Forray et al., 1994). Many adrenoceptor drugs developed before 1992 are not selective for any particular α-adrenoceptor subtype. It is increasingly evident that this lack of receptor subtype selectivity is an underlying cause of the untoward side-effects of these drugs.

The role of the sympathetic adrenergic nervous system in the storage function of the bladder is well recognized (Wein, 1987; Latifpour et al, 1990). Likewise, it is understood in the art that the study of adrenoceptor mechanisms in isolated urethra and bladder tissues is applicable to incontinence therapy (Latifpour et al., 1994; Tsujimoto et al., 1986). Various groups have attempted to identify, through binding and functional studies, α_1 receptor subtypes in the urethrae of humans, rabbits, and rats (Yoshida et al., 1991; Testa et al. 1993; Chess-Williams et al., 1994). These efforts have, thus far, failed to provide conclusive evidence for a

particular α_1-adrenoceptor subtype being responsible for the effects of adrenoceptor agonists in the urethra.

This invention relates to the discovery that α_{1C}-agonists are useful for the treatment of urinary incontinence with the potential for decreased side effects. Data already exists which indicates that the α_{1C}-adrenoceptor is not involved significantly in the cardiovascular actions of α-agonists and antagonists (Forray et al., 1994). Therefore, agonists exhibiting significant binding and functional selectivity for the α_{1C}-adrenoceptor over other α_1-adrenoceptors, α_2-adrenoceptors, β-adrenoceptors, as well as histamine receptors and serotonin (5-HT) receptors, are contemplated to be more effective agents, relative to currently available therapies, for the treatment of urinary incontinence.

Summary of the Invention

The present invention provides a method of treating urinary incontinence in a subject which comprises administering to the subject a therapeutically effective amount of an α_{1C} selective agonist which activates a human α_{1C} adrenoceptor at least ten-fold more than it activates a human α_{1A} adrenoceptor and a human α_{1B} adrenoceptor.

The present invention further provides a method of inducing contraction of urethra and bladder neck tissues which comprises contacting the urethra and bladder neck tissues with an effective contraction-inducing amount of an α_{1C} selective agonist which activates a human α_{1C} adrenoceptor at least ten-fold more than it activates a human α_{1A} adrenoceptor and a human α_{1B} adrenoceptor.

In addition, the invention includes compounds for the treatment of urinary incontinence and for use in inducing contraction of urethra and bladder neck tissues.

Brief Description of the Figures

Figures 1A, 1B, and 1C show correlation of antagonist pK_B values determined in functional studies of human urethra versus pK_I values measured in binding experiments using cloned human α_{1A}-adrenoceptors (A), α_{1B}-adrenoceptors (B), and α_{1C}-adrenoceptors (C). The slopes and correlation coefficients (r) for the linear regression analysis are presented in each figure.

Figures 2A, 2B, and 2C show correlation of antagonist pK_B values determined in functional studies of female rabbit urethra versus pK_I values measured in binding experiments using cloned human α_{1A}-adrenoceptors (A), α_{1B}-adrenoceptors (B), and α_{1C}-adrenoceptors (C). The slopes and correlation coefficients (r) for the linear regression analysis are presented in each figure.

Figures 3A, 3B, and 3C show correlation of antagonist pK_B values determined in functional studies of male rabbit urethra versus pK_I values measured in binding experiments using cloned human α_{1A}-adrenoceptors (A), α_{1B}-adrenoceptors (B), and $_{1C}$-adrenoceptors (C). The slopes and correlation coefficients (r) for the linear regression analysis are presented in each figure.

Figures 4A, 4B, and 4C show correlation of antagonist pK_B values determined in functional studies of female dog urethra versus pK_I values measured in binding experiments using cloned human α_{1A}-adrenoceptors (A), α_{1B}-adrenoceptors (B), and α_{1C}-adrenoceptors (C). The slopes and correlation coefficients (r) for the linear regression analysis are presented in each figure.

Figures 5, 5B, and 5C show correlation of antagonist pK_B

values determined in functional studies of male dog urethra versus pK_I values measured in binding experiments using cloned human α_{1A}-adrenoceptors (A), α_{1B}-adrenoceptors (B), and α_{1C}-adrenoceptors (C). The slopes and correlation coefficients (r) for the linear regression analysis are presented in each figure.

Figure 6 shows the chemical structures of SK&F 102652, A-61603, SDZ NVI 085, Prazosin, 5-Methyl urapidil, Abanoquil, Compound 1, and ST-1059.

Detailed Description of the Invention

The following definitions are presented as an aid in understanding this invention.

Receptor Activation describes the process in which the binding of a compound to the receptor when it is on the surface of a cell leads to a metabolic response within the cell. Such metabolic responses include, but are not limited to, activation of adenylyl cyclase, activation of guanylyl cyclase, hydrolysis of inositol phospholipids, movement of ions across the cell membrane, or contraction in a tissue in the cells of which the receptor is expressed.

Potency means the concentration of an agonist which elicits half of its maximum activation (expressed as EC_{50} or the negative log of the EC_{50}, i.e., pEC_{50}).

Intrinsic Activity means the magnitude of the maximum activation in a cell or tissue which a particular agonist is capable of eliciting, relative to the maximum activation elicited by a reference full agonist and is expressed as values ranging between unity for full agonists (e.g., norepinephrine in the case of α-adrenoceptors) and zero for antagonists. Because intrinsic activity as originally defined (Ariens, 1960) is recognized as being dependent upon the receptor system in which it is measured (Kenakin, 1987), intrinsic activity herein is based upon measurements made using the cloned receptor systems described below.

Selectivity of Receptor Activation refers to the ability of an agonist to selectively activate one receptor relative to another receptor. Such selectivity may

reflect either (a) the agonist's ability to activate one receptor at a much lower concentration than that required to activate another receptor (i.e., a potency difference) or (b) the agonist's ability to activate one receptor to a much greater degree than another receptor, independent of concentration, (i.e., an intrinsic activity difference) or (c) a combination of both.

Therefore, statements of the form "activates a human α_{1c}-adrenoceptor at least ten-fold more than it activates any of the following (receptors)" mean and include any such difference whether it is by virtue of a difference in potency, or a difference in intrinsic activity, or both.

Having due regard to the preceding definitions, the present invention provides a method of treating urinary incontinence in a subject which comprises administering to the subject a therapeutically effective amount of an α_{1C} selective agonist which activates a human α_{1C} adrenoceptor at least ten-fold more than it activates a human α_{1A} adrenoceptor and a human α_{1B} adrenoceptor.

The invention further provides a method of treating urinary incontinence in a subject which comprises administering to the subject a therapeutically effective amount of an α_{1C} selective agonist which activates a human α_{1C} adrenoceptor at least 50-fold more than it activates a human α_{1A} adrenoceptor and a human α_{1B} adrenoceptor.

The invention provides a method of treating urinary incontinence in a subject which comprises administering to the subject a therapeutically effective amount of an α_{1C} selective agonist which activates a human α_{1C} adrenoceptor at least 100-fold more than it activates a human α_{1A} adrenoceptor and a human α_{1B} adrenoceptor.

The invention provides a method of treating urinary incontinence in a subject which comprises administering to the subject a therapeutically effective amount of an α_{1C} selective agonist which activates a human α_{1C} adrenoceptor at least 200-fold more than it activates a human α_{1A} adrenoceptor and a human α_{1B} adrenoceptor.

The α_{1C} selective agonist used to practice the method of treating urinary incontinence further has the characteristic that it does not antagonize a human α_{1A} adrenoceptor and a human α_{1B} adrenoceptor.

Desirably, the α_{1C} selective agonist used to practice the method of treating urinary incontinence activates the human α_{1C} adrenoceptor at least ten-fold more than it activates any human α_2 adrenoceptor and any β adrenoceptor. Some examples of α_2 adrenoceptors include the α_{2A}, α_{2B}, and α_{2C} receptors.

The invention also provides that the α_{1C} selective agonist used to practice the method of treating urinary incontinence further has the characteristic that it does not antagonize any human α_2 adrenoceptor and any β adrenoceptor. Some examples of α_2 adrenoceptors include the α_{2A}, α_{2B}, and α_{2C} receptors.

Desirably, the α_{1C} selective agonist used to practice the method of treating urinary incontinence activates the human α_{1C} adrenoceptor at least ten-fold more than it activates a human histamine H_1 or H_2 receptor.

The invention further provides that the α_{1C} selective agonist used to practice the method of treating urinary incontinence activates the human α_{1C} adrenoceptor at least ten-fold more than it activates a human dopamine D_1, D_2,

D_3, or D_5 receptor.

The invention also provides that the α_{1C} selective agonist used to practice the method of treating urinary incontinence activates the human α_{1C} adrenoceptor at least ten-fold more than it activates a human serotonin 5-HT_{1A}, 5-$HT_{1D\alpha}$, 5-$HT_{1D\beta}$, 5-HT_{1E}, 5-HT_{1F}, 5-HT_2, or 5-HT_7 receptor.

The present invention further provides a method of inducing contraction of urethra and bladder neck tissues which comprises contacting the urethra and bladder neck tissues with an effective contraction-inducing amount of an α_{1C} selective agonist which activates a human α_{1C} adrenoceptor at least ten-fold more than it activates a human α_{1A} adrenoceptor and a human α_{1B} adrenoceptor.

The invention further provides a method of inducing contraction of urethra and bladder neck tissues which comprises contacting the urethra and bladder neck tissues with an effective contraction-inducing amount of an α_{1C} selective agonist which activates a human α_{1C} adrenoceptor at least 50-fold more than it activates a human α_{1A} adrenoceptor and a human α_{1B} adrenoceptor.

The invention provides a method of inducing contraction of urethra and bladder neck tissues which comprises contacting the urethra and bladder neck tissues with an effective contraction-inducing amount of an α_{1C} selective agonist which activates a human α_{1C} adrenoceptor at least 100-fold more than it activates a human α_{1A} adrenoceptor and a human α_{1B} adrenoceptor.

The invention also provides a method of inducing contraction of urethra and bladder neck tissues which comprises contacting the urethra and bladder neck tissues

with an effective contraction-inducing amount of an α_{1C} selective agonist which activates a human α_{1C} adrenoceptor at least 200-fold more than it activates a human α_{1A} adrenoceptor and a human α_{1B} adrenoceptor.

The α_{1C} selective agonist used to practice the method of inducing contraction of urethra and bladder neck tissues further has the characteristic that it does not antagonize a human α_{1A} adrenoceptor and a human α_{1B} adrenoceptor.

Desirably, the α_{1C} selective agonist used to practice the method of inducing contraction of urethra and bladder neck tissues activates the human α_{1C} adrenoceptor at least ten-fold more than it activates any human α_2 adrenoceptor and any β adrenoceptor. Some examples of α_2 adrenoceptors include the α_{2A}, α_{2B}, and α_{2C} receptors

The invention also provides that the α_{1C} selective agonist used to practice the method of inducing contraction of urethra and bladder neck tissues further has the characteristic that it does not antagonize any human α_2 adrenoceptor and any β adrenoceptor. Some examples of α_2 adrenoceptors include the α_{2A}, α_{2B}, and α_{2C} receptors

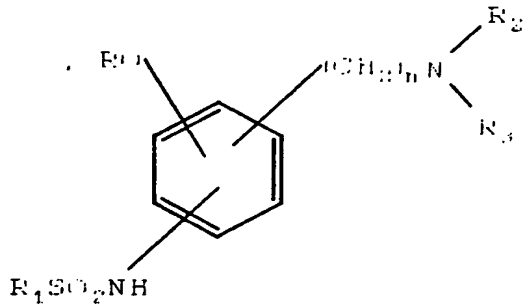
Desirably, the α_{1C} selective agonist used to practice the method of inducing contraction of urethra and bladder neck tissues activates the human α_{1C} adrenoceptor at least ten-fold more than it activates a human histamine H_1 or H_2 receptor.

The invention further provides that the α_{1C} selective agonist used to practice the method of inducing contraction of urethra and bladder neck tissues activates the human α_{1C} adrenoceptor at least ten-fold more than it

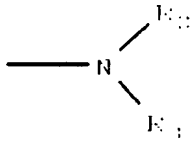
activates a human dopamine D_1, D_2, D_3, or D_5 receptor.

The invention also provides that the α_{1C} selective agonist used to practice the method of inducing contraction of urethra and bladder neck tissues activates the human α_{1C} adrenoceptor at least ten-fold more than it activates a human serotonin 5-HT_{1A}, 5-$HT_{1D\alpha}$, 5-$HT_{1D\beta}$, 5-HT_{1E}, 5-HT_{1F}, 5-HT_2, or 5-HT, receptor.

In one embodiment the invention provides a method of treating urinary incontinence which comprises administering to the subject a therapeutically effective amount of a compound having the structure:



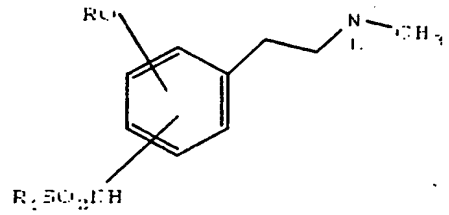
where n is an integer from 1 to 6; R is H or C_1-C_6 alkyl; R_1 is C_1-C_6 alkyl, phenyl, naphthyl, substituted phenyl or naphthyl where the substituent is a halogen, or a C_1-C_6 alkyl or alkoxy group; where



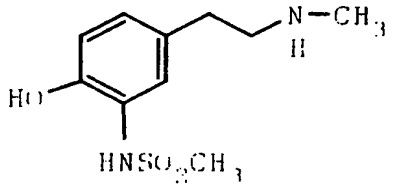
is an amino group or a heterocyclic group; the heterocyclic group is piperidine, morpholine, piperazine, pyrrolidine, hexamethylene, or thiomorpholine, the

heterocyclic group being bonded through the nitrogen atom thereof to the $(CH_2)_n$ group; the amino group, where R_2 is H, C_1-C_6 alkyl, benzyl, or benzyhydryl and where R_3 is H; C_1-C_{10} alkyl; C_2-C_{10} alkenyl; C_3-C_{10} cycloalkyl or cycloalkenyl.

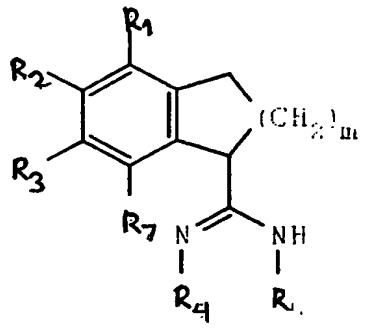
The present invention also provides that the compound has the structure:



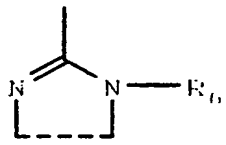
The invention further provides that the compound has the structure:



A further embodiment of the invention provides a method of treating urinary incontinence which comprises administering to the subject a therapeutically effective amount of a compound having the structure:

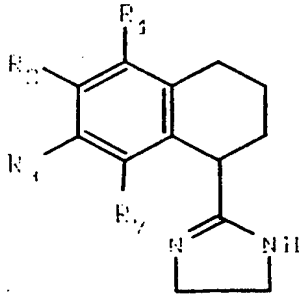


where m is an integer from 0 to 2; where each of R_1, R_2, R_3 and R_7 is independently H; OH; C_1-C_6 alkyl or alkoxy; halo; amino; acetamido or $NHSO_2R$ with R being H or C_1-C_6 alkyl; where R_1 and R_2 or R_2 and R_3 or R_3 and R_7 taken together constitute a methylenedioxy, ethylenedioxy, benzimidazole or indole ring; where each of R_4 and R_5 are independently H or taken together has the following formula:

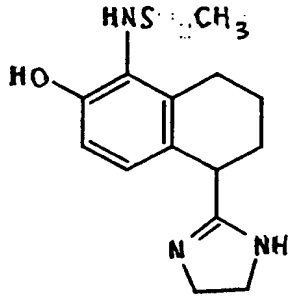


where the dashed line represents a single or double bond; and R_6 is H or C_1-C_6 alkyl; or a pharmaceutically acceptable salt thereof.

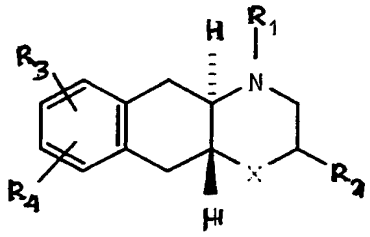
The invention also provides that the compound has the structure:



The invention further provides that the compound has the structure:

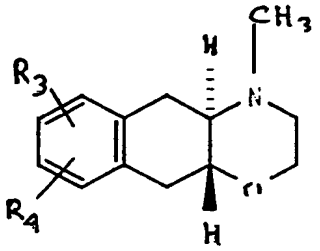


The invention also provides a method of treating urinary incontinence which comprises administering to the subject a therapeutically effective amount of a compound having the structure:

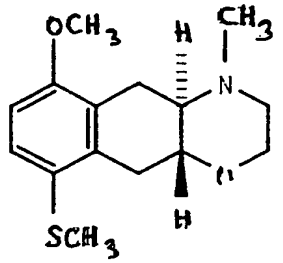


where each of R_1 and R_2 is independently H or C_1-C_4 alkyl; where R_3 is OH or C_1-C_4 alkoxy; and R_4 is C_1-C_4 alkylthio, alkylsulfoxide or alkylsulfone; Cl; Br; I or CF_3; where X is O, S, SO, SO_2, NH, NR_1 or $NC(O)R_1$; in free base or acid addition salt form.

The invention further provides that the compound has the structure:



The invention specifically provides that the compound has the structure:



This invention is also directed to optical isomers of the compounds described above. The invention also provides for the (-) and (+) enantiomers of all compounds of the subject application described herein. Included in this invention are pharmaceutically acceptable salts and complexes of all of the compounds described herein. The salts include but are not limited to the following acids and bases. The following inorganic acids; hydrochloric acid, hydrofluoric acid, hydrobromic acid, hydroiodic acid, sulfuric acid and boric acid. The organic acids; acetic acid, trifluoroacetic acid, formic acid, oxalic acid, malonic acid, succinic acid, fumaric acid, tartaric acid, maleic acid, citric acid, methanesulfonic acid, trifluoromethanesulfonic acid, benzoic acid, glycolic acid, lactic acid and mandelic acid. The following

inorganic bases; ammonia, hydroxyethylamine and hydrazine. The following organic bases; methylamine, ethylamine, propylamine, dimethylamine, diethylamine, trimethylamine, triethylamine, ethylenediamine, hydroxyethylamine, morpholine, piperazine and guanidine. This invention further provides for the hydrates, isomorphs and polymorphs of all of the compounds described herein.

The present invention therefore provides a method of treating urinary incontinence, which comprises administering a quantity of any of the α_{1C} receptor agonists defined herein in a quantity effective against urinary incontinence.

The drug may be administered to a patient afflicted with urinary incontinence by any conventional route of administration, including, but not limited to, intravenous, intramuscular, oral, subcutaneous, intratumoral, intradermal, and parenteral. The quantity effective against urinary incontinence is between 0.001 mg and 10.0 mg per kg of subject body weight. The method of treating urinary incontinence disclosed in the present invention may also be carried out using a pharmaceutical composition comprising any of the α_{1C} receptor agonists as defined herein and a pharmaceutically acceptable carrier. The composition may contain between 0.05 mg and 500 mg of an α_{1C} receptor agonist, and may be constituted into any form suitable for the mode of administration selected. Compositions suitable for oral administration include solid forms, such as pills, capsules, granules, tablets, and powders, and liquid forms, such as solutions, syrups, elixirs, and suspensions. Forms useful for parenteral administration include sterile solutions, emulsions, and suspensions.

The drug may otherwise be prepared as a sterile solid composition which may be dissolved or suspended at the time of administration using sterile water, saline, or other appropriate sterile injectable medium. Carriers are intended to include necessary and inert binders, suspending agents, lubricants, flavorants, sweeteners, preservatives, dyes, and coatings. The drug may also be formulated as a transdermal patch.

Optimal dosages to be administered may be readily determined by those skilled in the art, and will vary with the particular α_{1c} receptor agonist in use, the strength of the preparation, the mode of administration, and the advancement of the disease condition. Additional factors depending on the particular patient being treated will result in a need to adjust dosages, including patient age, weight, diet, and time of administration.

The term "therapeutically effective amount" as used herein refers to that amount of pharmaceutical agent that elicits in a tissue, system, animal or human, the biological or medicinal response that is being sought by a researcher, veterinarian, medical doctor or other clinician, which response includes alleviation of the symptoms of the disease being treated. The term "subject," as used herein refers to an animal, preferably a mammal, most preferably a human, who has been the object of treatment, observation or experiment.

The binding and functional properties of compounds at the different human receptors were determined *in vitro* using cultured cell lines that selectively express the receptor of interest. These cell lines were prepared by transfecting the cloned cDNA or cloned genomic DNA or constructs containing both genomic DNA and cDNA encoding

the human α-adrenoceptors as further described in detail in Example 10 hereinbelow. In connection with this invention, a number of cloned human receptors discussed herein, either as plasmids or as stably transfected cell lines, have been made pursuant to, and in satisfaction of, the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purpose of Patent Procedure, and are made with the American Type Culture Collection, 12301 Parklawn Drive, Rockville, Maryland 20852. Specifically, these deposits have been accorded ATCC Accession Numbers as follows in Table 1:

Table 1 - ATCC Deposits:

Designation	Receptor	ATCC Accession No.	Date of Deposit
Cell lines:			
L-α_{1A}	human α_{1A}	CRL 11138	09/25/1992
L-α_{1B}	human α_{1B}	CRL 11139	09/25/1992
L-α_{1C}	human α_{1C}	CRL 11140	09/25/1992
L-α_{2A}	human α_{2A}	CRL 11180	11/6/1992
L-NGC-α_{2B}	human α_{2B}	CRL 10275	10/25/1989
Y-a2B-2	human α_{2B}	CRL 11888	05/11/1995
L-α_{2C}	human α_{2C}	CRL 11181	11/6/1992
Ltk-8-30-84	human 5-HT_{1D1}	CRL 10421	04/17/1990
Ltk-11	human 5-HT_{1D2}	CRL 10422	04/17/1990
$5HT_{1E}$-7	human 5-HT_{1E}	CRL 10913	11/6/1991
L-5-HT_{1F}	human 5-HT_{1F}	CRL 10957	12/27/1991
L-5HT-4B	human 5-HT_{4B}	CRL 11166	10/20/1992
5HT1A-3	human 5-HT_{1A}	CRL 11889	05/11/1995
L-NGC-$5HT_2$	human 5-HT_2	CRL 10287	10/31/1989
Plasmids:			
pcEXV-D2	human D2	75344	11/6/1992
pcEXV-H2	human H2	75345	11/6/1992
pcEXV-H1	human H1	75346	11/6/1992

Cell transfections

Transient transfections of COS-7 cells with various plasmids were performed using the DEAE-Dextran method. which is well-known to those skilled in the art. Briefly, a plasmid comprising an expression vector for the receptor of interest was added to monolayers of COS-7 cells bathed in a DEAE-Dextran solution. In order to enhance the efficiency of transfection, dimethyl sulfoxide was typically also added, according to the method of Lopata (Lopata, et al., 1984). Cells were then grown under controlled conditions and used in experiments after about 72 hours.

Stable cell lines were obtained using means which are well-known in the art. For example, a suitable host cell was typically cotransfected, using the calcium phosphate technique, with a plasmid comprising an expression vector for the receptor of interest and a plasmid comprising a gene which allows selection of successfully transfected cells. Cells were then grown in a controlled environment, and selected for expression of the receptor in interest. By continuing to grow and select cells, stable cell lines were obtained expressing the receptors described and used herein.

Binding assays

The binding of a test compound to a receptor of interest was generally evaluated by competitive binding assays using membrane preparations derived from cells which expressed the receptor. First, conditions were determined which allowed measurement of the specific binding of a compound known to bind to the receptor. Then, the binding of the known compound to the receptor

in membrane preparations was evaluated in the presence of several different concentrations of the test compound. Binding of the test compound to the receptor resulted in a reduction in the amount of the known compound which was abound to the receptor. A test compound having a high affinity for the receptor of interest would displace a given fraction of the bound known compound at a concentration lower than the concentration which would be required if the test compound had a low affinity for the receptor of interest.

The data shown in the Table 2 indicate that it is the α_{1c}-adrenoceptor which is responsible for mediating the contractile response to adrenoceptor agonists in the urethra of mammals, particularly humans. This *in vitro* property is recognized in the art as correlating with efficacy in treating urinary incontinence *in vivo*.

This invention will be better understood from the Experimental Details which follow. However, one skilled in the art will readily appreciate that the specific methods and results discussed are merely illustrative of the invention as described more fully in the claims which follow thereafter.

Experimental Details

Phenylephrine, prazosin, 5-methylurapidil, and BMY 7378 were obtained from Research Biochemicals, Inc. Other compounds were prepared according to the examples which follow.

EXAMPLE 1

Synthesis of (±)-N-[5-(4,5-Dihydro-1H-imidazol-2-yl)-2-hydroxy-5,6,7,8-tetra-hydronaphthalen-1-

yl]methanesulfonamide (A-61603) 5-Nitro-6-methoxy-1-tetralone. To a solution of 100 ml of 70% HNO_3 was added 6-methoxytetralone (Aldrich Chemical Co., Milwaukee, Wisconsin, 4.0 g, 23 mmol) over 1 h period at 0 °C. The resulting solution was stirred for 24 h at 25 °C. The reaction mixture was then poured into water to yield a yellow precipitate, which was subjected to column chromatography (40% EtOAc-Hexane) to yield 2.2 g (43%) of the desired product. ^1H NMR (300 MHz, $CDCl_3$): δ 2.12 (qt, 2H, J=6.9 Hz), 2.61 (t, 2H, J=6.9 Hz), 2.83 (t, 2H, J=6.9 Hz), 3.93 (s, 3H), 6.98 (d, 1H, J=8.9 Hz), 8.14 (d,1H, J=8.9 Hz).

Synthesis of (±)-6-Methoxy-5-nitro-1-trimethylsilyloxy-1,2,3,4-tetrahydronaph-thalene-1-carbonitrile. To a solution of 5-nitro-6-methoxy-1-tetralone (0.93 g, 4.2 mmol) in 20 ml of CH_2Cl_2 was added ZnI_2 (100 mg, 0.31 mmol) and TMSCN (0.84 ml, 6.3 mmol) and the resulting solution was stirred for 2 h at 25 °C. The reaction mixture was concentrated *in vacuo* to provide the desired product as a colorless oil, which was used in the next step without purification. ^1H NMR (300 MHz, $CDCl_3$):δ 0.028 (s, 9H), 1.88-2.35 (m, 4H), 2.67 (t, 2H, J=6.1 Hz), 3.88 (s, 3H), 6.97 (d, 1H, J=8.9 Hz), 7.71 (d, 1H, J=8.9 Hz).

Synthesis of (±)-6-Methoxy-5-nitro-3,4-dihydronaphthalene-1-carbonitrile. A solution of 6-methoxy-5-nitro-1-trimethylsilyloxy-1,2,3,4-tetrahydronaphthalene-1-carbonitrile (1.3 g, 4.0 mmol) and AcCl (1.0 ml) in 20 ml AcOH was stirred for 2 h at 80-100 °C. The resulting reaction mixture was concentrated *in vacuo* to yield the desired product as a colorless oil (0.86 g, 4.0 mmol, 96% for two steps), which was subjected to the following step without any

further purification. ^{1}H NMR (300 MHz, $CDCl_3$):δ 2.48 (dt, 2H, J=2.3, 6.7 Hz), 2.78 (t, 2H, J=8.9 Hz), 3.89 (s, 3H), 6.82 (t, 1H, J=2.3 Hz), 6.92 (d, 1H, J=8.9 Hz), 7.53 (d, 1H, J=8.9 Hz).

Synthesis of (±)-6-Methoxy-5-nitro-1,2,3,4-tetrahydronaphthalene-1-carbonitrile. To a solution of 6-methoxy-5-nitro-3,4-dihydronaphthalene-1-carbonitrile (0.41 g, 1.8 mmol) in 10 ml of EtOH was added $NaBH_4$ (0.20 g, 5.3 mmol) and the resulting reaction mixture was stirred for 30 min at 25 °C. The solvent was removed *in vacuo* to yield an oily residue which was dissolved in EtOAc and washed with brine. The organic layer was dried over Na_2SO_4 and concentrated *in vacuo* to yield the desired product as a colorless oil (0.42 g, >95%) which was subjected to a following reaction without purification. ^{1}H NMR (300 MHz, $CDCl_3$):δ 1.86 (m ,2H), 2.04 (m, 2H), 2.62 (q, 2H, J=6.3 Hz), 3.83 (s, 3H), 4.17 (t, 1H, J=6.3 Hz), 7.10 (d, 1H, J=8.9 Hz), 7.45 (d, 2H, J=8.9 Hz).

Synthesis of (±)-6-Methoxy-5-amino-1,2,3,4-tetrahydronaphthalen-1-carbonitrile. A solution of 6-methoxy-5-nitro-1,2,3,4-tetrahydronaphthalene-1-carbonitrile (0.42 g, 1.8 mmol) and catalytic amount of 10% Pd/C in 100 ml of MeOH was stirred under H_2 for 12 h at 25 °C. The reaction mixture was filtered and concentrated *in vacuo* to yield the desired product as a colorless oil (0.36 g, >95%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.93 (m, 4H), 2.48 (qt, 2H, J=5.7Hz), 3.79 (s, 3H), 3.99 (t, 1H, J=6.3 Hz), 6.68 (dd, 2H, J=8.0, 8.9 Hz).

Synthesis of (±)-N-(5-Cyano-2-methoxy-5,6,7,8-tetrahydronaphthalen-1-yl)methane-sulfonamide. To a solution of 6-methoxy-5-amino-1,2,3,4-tetrahydronaphthalene-1-carbonitrile (1.7 g, 8.3 mmol) in

20 ml of anhydrous pyridine was added methanesulfonyl chloride (1.9 ml, 1.2 mmol). The resulting solution was stirred for 2 h at 25 °C. The reaction mixture was concentrated *in vacuo* to yield an oily residue which was redissolved in EtOAc and washed with aqueous $NaHCO_3$. The organic layer was dried over Na_2SO_4 and concentrated *in vacuo* to provide an oily residue which was purified by column chromatography (EtOAc, neat) to yield 1.5 g (65%) of the desired product. ^1H NMR (300 MHz, $CDCl_3$): δ 1.82 (m,1H), 1.94 (m, 1H), 2.14 (m, 2H), 2.96 (s, 3H), 3.03 (q, 2H, J=8.8 Hz), 3.84 (s, 3H), 3.96 (t, 1H, J=6.3 Hz), 6.85 (d, 1H, J=8.9 Hz), 7.39 (d, 1H, J=8.9 Hz).

Synthesis of (±)-N-[5-(4,5-Dihydro-1H-imidazol-2-yl)-2-methoxy-5,6,7,8-tetra-hydronaphthalen-1-yl]methanesulfonamide. N-(5-Cyano-2-methoxy-5,6,7,8-tetrahydronaphthalen-1-yl)methane-sulfonamide (1.5 g, 5.4 mmol) was dissolved in 200 ml of MeOH and cooled to 0 °C. The solution was then treated with dry HCl gas for 2 h, sealed tightly and stored for 12 h at 25 °C. The solvent was removed and the residue was redissolved in 100 ml of MeOH, followed by addition of ethylenediamine (0.67 ml, 10 mmol). The resulting solution was stirred at reflux for 12 h. The reaction mixture was concentrated *in vacuo*, yielding an oily residue which was subjected to column chromatography (25% NH_3 sat'd MeOH-$CHCl_3$) to provide 1.3 g (76%) of the desired product. ^1H NMR (300 MHz, CD_3OD): δ 1.78 (m, 2H), 1.96 (m, 1H), 2.12 (m, 1H), 2.95 (broad t, 2H), 2.98 (s, 3H), 3.82 (s, 3H), 3.90 (broad s, 4H), 4.11 (t, 1H, J=6.3 Hz), 6.96 (t, 1H, J=8.9 Hz), 7.05 (t, 1H, J=8.9 Hz).

Synthesis of (±)-N-[5-(4,5-Dihydro-1H-imidazol-2-yl)-2-hydroxy-5,6,7,8-tetra-hydronaphthalen-1-yl]methanesulfonamide (A-61603). To a solution of N-[5-

(4,5-dihydro-1H-imidazol-2-yl)-2-methoxy-5,6,7,8-tetrahydronaphthalen-1-yl]methanesulfonamide (0.3 g, 0.9 mmol) in 100 ml of $CHCl_3$ was added BBr_3 (2.0 ml, 2.0 mmol) at -78 °C. The resulting reaction mixture was stirred for 12 h at 25 °C. The reaction mixture was then recooled to -78 °C and 2 ml of MeOH was added. The reaction mixture was warmed to 25 °C and stirred for another 3 h. It was then concentrated *in vacuo* to provide a light yellow solid (0.35 g, >95%) which was identified as the HBr salt of the desired product, mp 263-265 °C. ^1H NMR (300 MHz, CD_3OD): δ 1.78 (m, 2H), 1.90 (m, 1H), 2.04 (m, 1H), 2.93 (broad t, 2H), 3.05 (s, 3H), 3.86 (s, 4H), 4.06 (t, 1H, J=6.3 Hz), 6.79 (d, 1H, J=8.9 Hz), 6.92 (d, 1H, J=8.9 Hz), 9.58 (s, 1H); Anal. Cal. For $C_{14}H_{19}N_3O_3S.1.0HBr$ requires C, 43.7; H, 5.12; N, 10.7. Found: C, 42.8; H, 4.95; N, 10.3.

EXAMPLE 2

Synthesis of N-[2-Hydroxy-5-[2-methylamino)ethyl]phenyl]-methanesulfonamide (SK&F 102652)4-Hydroxy-N-methyl-3-nitrobenzeneacetamide. A mixture of 5 g (25.38 mmol) of 3-nitro-4-hydroxyphenylacetic acid (from Aldrich Chemical Co., Milwaukee, Wisconsin) in 20 mL of thionyl chloride was heated at reflux for 45 min. The reaction mixture was cooled and poured into 80 mL of hexane. The resulting precipitate was collected by filtration, washed with hexane, and air-dried to yield a yellow solid. A solution of this acid chloride in 100 mL of dichloromethane was cooled in an ice bath and stirred while excess methylamine was distilled in dropwise. The mixture was stirred at room temperature overnight. The precipitated solid was collected by filtration and dissolved in water. It was acidified to pH 2 with 3 N HCl and extracted with dichloromethane to yield a yellow

solid 4.2 g(80%). The title compound was used in the next step without further purification. ^1H NMR (300 MHz, $CDCl_3$) δ 10.50 (s, 1H), 7.97 (s, 1H), 7.50 (d, 1H, J = 9.0 Hz), 7.11 (d, 1H, J = 9.0 Hz), 5.4 (brs, 1H), 3.49 (s, 2H), 2.78 (d, 3H, J = 6.0 Hz).

Synthesis of 4-Methoxy-N-methyl-3-nitrobenzeneacetamide. To a solution of 4.2 g (19.8 mmol) of 4-hydroxy-N-methyl-3-nitrobenzeneacetamide in 50 mL of DMF containing 5.5 g of anhydrous potassium carbonate was added 5.6 mL of dimethyl sulfate. The mixture was heated at 60-70 °C for 45 min, treated with an additional 3.0 mL of methyl sulfate, and heated for another 30 min. The mixture was cooled, poured into 200 mL water, and extracted with dichloromethane. The extracts were washed with water, dried and evaporated to give a solid which was recrystallized from ethanol-water to afford 3.7 g of a yellowish solid (82%). ^1H NMR (300 MHz, $CDCl_3$) δ 7.71 (s, 1H), 7.47 (d, 1H, J = 9.0 Hz), 7.25 (d, 1H, J = 8.4 Hz), 5.71 (brs, 1H),3.90 (s, 3H) 3.47 (s, 2H), 2.75 (d, 3H, J = 4.8 Hz).

Synthesis of 4-Methoxy-N-methyl-3-[(methylsulfonyl)amino]-benzeneacetamide. A solution of 3.6 g (16.3 mmol) of 4-methoxy-N-methyl-3-nitrobenzeneacetamide was hydrogenated using hydrogen gas at 50 psi in 40 mL of ethanol over 200 mg of Pd/C (10%) for 6 h. The catalyst was removed by filtration and solvent was evaporated to give 2.9 g of white solid. This solid was dissolved in 30 mL of pyridine and treated dropwise with 1.5 mL (19.4 mmol) of methanesulfonyl chloride in 5 mL of pyridine. The reaction mixture was warmed to 65 °C for 30 min and then stirred at room temperature overnight. The pyridine was evaporated and the residue taken up in 40 mL of water, adjusted to pH

6.7, and cooled in an ice bath. The resulting precipitate was removed by filtration and dried to give 1.7 g of an off-white solid(42%). Recrystallization from methanol gave white crystals. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.35 (s, 1H), 7.04 (d, 1H, J = 9.0 Hz), 6.85 (d, 1H, J = 8.4 Hz),6.78 (brs, 1H),5.41 (brs, 1H), 3.85 (s, 3H) 3.46 (s, 2H),2.94 (s, 3H), 2.73 (d, 3H, J = 4.8 Hz).

Synthesis of N-[2-Methoxy-5-[2-methylamino)ethyl]phenyl]-methanesulfonamide. A solution of 0.8 g (2.89 mmol) of 4-methoxy-N-methyl-3-[(methylsulfonyl)amino]-benzeneacetamide in 20 ml of dry THF was stirred and cooled in ice as a 1 M solution of borane in THF (15 mL) was added dropwise. After the addition was complete, the mixture was warmed to 65 °C for 6 h. It was cooled and treated with 25 mL of methanol, followed by 1 mL of 6 N HCl . The mixture was evaporated to yield a white residue which was dissolved in a minimum amount of hot methanol, filtered, and treated with ethyl acetate until cloudy and allowed to crystallize. The solid was removed by filtration and dried to give 0.54 g of white crystals (72%). ^{1}H NMR (300 MHz, CD_3OD) δ 7.28 (s, 1H), 7.07 (d, 1H, J = 9.0 Hz), 7.00 (d, 1H, J = 8.4 Hz), 3.83 (s, 3H) 3.19-3.14 (m, 2H),2.90-2.85(m, 2H), 2.86 (s, 3H) 2.66 (s, 3H,).

Synthesis of N-[2-Hydroxy-5-[2-methylamino)ethyl]phenyl]-methanesulfonamide (SK&F 102652). A suspension of 0.2 g of N-[2-methoxy-5-[2-methylamino)ethyl]phenyl]-methanesulfonamide in 10 mL of dichloromethane in a dry ice-2-propanol bath was treated with 4 mL of 1 M BBr_3 in dichloromethane. It was allowed to warm to room temperature and stirred overnight. The mixture was treated with 50 mL of methanol, stirred for 1 h, evaporated , and treated again with methanol, and

evaporated to dryness. This residue was taken up in minimum volume of hot methanol, treated with ethyl acetate, and allowed to crystallize to afford 0.17 g (67%) of tan crystals, mp 188-189 °C. ^{1}H NMR (300 MHz, CD_3OD) δ 7.23 (s, 1H), 6.97 (d, 1H, J = 9.0 Hz), 6.85 (d, 1H, J = 8.4 Hz), 3.21-3.16 (m, 2H), 2.92 (s, 3H) 2.90-2.84(m, 2H), 2.68 (s, 3H,). Anal. Calcd for $C_{10}H_{17}BrN_2O_3S.0.05\ CH_2Cl_2$: C, 36.69; H, 5.23; N, 8.50. Found: C, 36.57; H, 5.14; N, 8.26.

EXAMPLE 3

Synthesis of (±)-4-Methyl-6-methoxy-9-thiomethoxy-3,4,4a,5,10,10a-hexahydro-2H-naphtho[2,3-b][1,4]-oxazine (SDZ NVI 085)1,4 Dihydro-5-methoxynaphthalene. To a refluxing solution of 1-methoxynaphthalene (Aldrich Chemical Co., Milwaukee, Wisconsin, 5.5 g, 34 mmol) in 80 ml of EtOH was added sodium (5.7 g, 250 mmol) in pieces under Ar. When all of the sodium was consumed, the reaction was cooled to 25 °C and stirred for additional 3 h. The reaction mixture was carefully quenched by adding 100 ml of water and extracted with EtOAc. The organic layer was dried over Na_2SO_4 and concentrated *in vacuo* to yield an oily residue which was purified by column chromatography (30% EtOAc-Hexane) to yield 2.8 g (52%) of the desired product as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.27(m, 2H), 3.39 (m, 2H), 3.82 (s, 3H), 5.85 (m, 2H), 6.67-6.75 (m, 2H), 7.13 (t, 1H, J=7.9 Hz).

Synthesis of (±)-6-Methoxy-1a,2,7,7a-tetrahydro-1-oxa-cyclopropan[b]naphthalene. To a solution of 1,4 dihydro-5-methoxynaphthalene (2.8 g, 17.5 mmol) in 50 ml of CH_2Cl_2 was added MCPBA (8.5 g, 50 mmol) in one portion. The resulting solution was stirred for 3 h at 0 °C . The

reaction mixture was diluted with CH_2Cl_2 and poured into a mixture of ice (50 g) and $NaHCO_3$ sat'd aqueous solution (150 ml). The organic layer was separated and the aqueous layer was extracted with CH_2Cl_2. The combined organic layers were washed with aqueous $NaHCO_3$, dried over $MgSO_4$ and concentrated *in vacuo* to yield an oil which was subjected to column chromatography (CH_2Cl_2, neat) to yield 1.7 g (59%) of the desired product as a colorless oil. ^1H NMR (300 MHz, $CDCl_3$): δ 2.78 (d, 1H, J=18.2 Hz), 3.21(AB q, 2H, J=18.3 Hz), 3.47 (m, 3H), 3.77 (s, 3H), 6.66(m, 2H), 7.08 (t, 1H, J=7.8 Hz).

Synthesis of (±)-3-Azido-5-methoxy-1,2,3,4-tetrahydronaphthalen-2-ol. To a solution of 6-methoxy-1a,2,7,7a-tetrahydro-1-oxa-cyclo-propan[b]naphthalene (1.7 g, 9.7 mmol) in 20 ml of DMSO was added sodium azide (5.6 g, 86 mmol) and H_2SO_4 (0.2 ml). The resulting suspension was stirred for 17 h at 25 °C. The reaction mixture was diluted with EtOAc and washed with brine. The organic layer was dried over $MgSO_4$ and concentrated *in vacuo*, yielding an oil which was subjected to column chromatography (EtOAc, neat) to provide 1.9 g (89%) of a mixture of two regioisomers in a 1:1 ratio. Two isomers were separated by fractional recrystallization in hexane to provide 0.6 g of the desired product, mp 83-84 °C. ^1H NMR (300 MHz, $CDCl_3$): δ 1.61 (s, 1H), 2.60 (dd, 1H, J=10.6, 17.0 Hz), 2.84 (dd, 1H, J=10.1, 15.9 Hz), 3.19 (dd, 1H, J=5.3, 15.9 Hz), 3.37 (dd, 1H, J=5.8, 17.0 Hz), 3.70 (m, 1H), 3.84 (s, 3H), 3.88 (m, 1H), 6.70(m, 2H), 7.17(t, 1H, J=7.8 Hz).

Synthesis of (±)-3-Amino-5-methoxy-1,2,3,4-tetrahydronaphthalen-2-ol. A solution of 3-azido-5-methoxy-1,2,3,4-tetrahydronaphthalen-2-ol (1.8 g, 8.0 mmol) in 150 ml of MeOH was stirred with 10% Pd/C (20 mg)

under H_2 (18 psi) for 4 h. The reaction mixture was filtered and concentrated *in vacuo* to provide 1.4 g (91%) of the desired product as a colorless oil which was used in the next reaction without purification. ^1H NMR (300 MHz, $CDCl_3$): δ 2.0 (broad s, 2H), 2.24 (dd, 1H, J=10.4, 16.8 Hz), 2.84 (m, 2H), 3.16 (m, 2H), 3.59 (m, 1H), 3.78 (s, 3H), 6.66 (m, 2H), 7.09 (t, 1H, J=7.9 Hz).

Synthesis of (±)-6-Methoxy-4a,5,10,10a-tetrahydro-4H-naphtho[2,3-b][1,4]oxazin-3-one. To a solution of 3-amino-5-methoxy-1,2,3,4-tetrahydronaphthalen-2-ol (1.7g, 8.8 mmol) and triethylamine (1.5 ml, 11 mmol) in 100 ml of CH_2Cl_2 was added chloroacetyl chloride (1.0 g, 8.9 mmol) in 10 ml of CH_2Cl_2 dropwise at 0 °C. The resulting solution was stirred for 1.5 h at 25 °C. The reaction mixture was then diluted with EtOAc and washed with 1N aqueous HCl. Organic layer was dried over Na_2SO_4 and concentrated *in vacuo*, yielding an oil which corresponds to the amide. The oily residue was redissolved in 20 ml of THF, and NaH (0.35 g, 8.8 mmol) and tetrabutylammonium iodide (0.25 g, 0.67 mmol) were added into the solution at 0 °C. The reaction mixture was stirred for 12 h at 25 °C. It was diluted with EtOAc and washed with brine. The organic layer was dried over Na_2SO_4 and concentrated *in vacuo*, yielding an oily residue which was purified by column chromatography (50% CH_2Cl_2-EtOAc) to provide 1.4 g (68%) of the desired product. ^1H NMR (300 MHz, $CDCl_3$): δ 2.45 (dd, 1H, J=10.5, 16.7 Hz), 2.85 (m, 1H), 3.11-3.34 (m, 2H), 3.65(m, 2H), 3.79 (s, 3H), 4.28 (AB q, 2H, 16.7 Hz), 6.68 (m, 2H), 7.07 (t, 1H, J=7.9 Hz).

Synthesis of (±)-6-Methoxy-3,4,4a,5,10,10a-hexahydro-2H-naphtho[2,3-b][1,4]oxazine. To a solution of 6-methoxy-4a,5,10,10a-tetrahydro-4H-naphto[2,3-b][1,4]oxazin-3-one (1.4 g, 6.0 mmol) in 100 ml of THF was added 10 ml of

$LiAlH_4$ solution in THF (10 mmol). The resulting solution was stirred for 2 h at reflux. The reaction was quenched with ice and the reaction mixture was then diluted with EtOAc. Filtration of the reaction mixture provided a clean organic layer which was concentrated *in vacuo* to yield 1.2 g (92%) of the desired product as a colorless oil. ^1H NMR (300 MHz, $CDCl_3$): δ 1.85 (s, 1H), 2.34(dd, 1H, J=12.0, 17.0 Hz), 2.78-3.16 (m, 6H), 3.50 (ddd, 1H, J=5.2, 6.8, 10.5 Hz), 3.74 (m, 1H), 3.81 (s, 3H), 3.94 (m, 1H), 6.70 (m, 2H), 7.16 (t, 1H, J=7.6 Hz).

Synthesis of (±)-6-Methoxy-3,4,4a,5,10,10a-hexahydro-2H-naphtho[2,3-b][1,4]oxazine-4-carboxylic acid benzyl ester. To a solution of 6-methoxy-3,4,4a,5,10,10a-hexahydro-2H-naphtho-[2,3-b][1,4]oxazine (0.62 g, 2.7 mmol) in 10 ml of CH_2Cl_2 was added triethylamine (1.0 ml, 7.2 mmol) and benzyl chloroformate (0.6 ml, 4.1 mmol). The resulting mixture was stirred at 25 °C for 3 h. It was then diluted with 100 ml of EtOAc and washed with brine. The organic layer was dried over Na_2SO_4 and concentrated *in vacuo*, yielding an oily residue which was subjected to column chromatography (20% EtOAc-CH_2Cl_2) to provide 0.46 g (48%) of the desired product. ^1H NMR (300 MHz, $CDCl_3$): δ 2.45 (dd, 1H, J=10.6, 16.6 Hz), 2.83 (dd, 1H, J=10.3, 16.0 Hz), 3.04 (dd, 1H, J=5.0, 16.0 Hz), 3.68-3.88 (m, 6H), 3.76 (s, 3H), 4.02(m, 1H), 5.16 (AB q, 2H, J=17.6 Hz), 6.66 (m, 2H), 7.09 (t, 1H,J=7.8 Hz).

Synthesis of (±)-4-Methyl-6-methoxy-3,4,4a,5,10,10a-hexahydro-2H-naphtho[2,3-b][1,4]oxazine. To a solution of 6-methoxy-3,4,4a,5,10,10a-hexahydro-2H-naphtho-[2,3-b][1,4]oxazine-4-carboxylic acid benzyl ester (0.40 g, 1.1 mmol) in THF was added 2.8 ml of $LiAlH_4$ solution(1.0 M)in THF. The resulting solution was stirred at reflux for 3 h. The reaction mixture was quenched with ice,

diluted with EtOAc and filtered through Celite. The organic layer was dried over Na_2SO_4 and concentrated *in vacuo*, yielding an oily residue which was subjected to column chromatography (5% MeOH-EtOAc) to yield 0.19 g (75%) of the desired product. ^1H NMR (300 MHz, $CDCl_3$): δ 2.07 (m, 1H), 2.28 (m, 1H), 2.37 (s, 3H), 2.49 (m, 1H), 2.77 (m, 2H), 2.97 (dd, 1H, J=5.5, 16.1 Hz), 3.28 (dd, 1H, J=5.5, 17.0 Hz), 3.55 (m, 1H), 3.79 (s, 3H), 3.86 (m, 2H), 6.67 (m, 2H), 7.09 (t, 1H, J=7.8 Hz).

Synthesis of (±)-4-Methyl-6-methoxy-9-iodo-3,4,4a,5,10,10a-hexahydro-2H-naphto-[2,3-b][1,4]oxazine. A solution of 4-methyl-6-methoxy-3,4,4a,5,10,10a-hexahydro-2H-naphtho[2,3-b][1,4]oxazine (0.37 g, 1.6 mmol) was dissolved in 7 ml of AcOH and heated to 50 °C. To the solution of the amine was added a solution of $Hg(OAc)_2$ (0.62 g, 19 mmol) and I_2 (1.0 g, 3.8 mmol) in 30 ml of AcOH. The resulting solution was stirred for 1 h at 50 °C and 1.5 h at 25 °C. The reaction mixture was filtered to remove mercury salts and concentrated *in vacuo*, yielding an oily residue which was subjected to column chromatography (5% NH_3 sat'd MeOH-EtOAc) to yield 0.25 g (44%) of the desired product. ^1H NMR (300 MHz, $CDCl_3$): δ 2.04 (m, 1H), 2.32 (dd, 1H, J=11.0, 18.0 Hz), 2.41 (s, 3H), 2.49 (m, 1H), 2.62 (dd, 1H, J=11.0, 17.0 Hz), 2.76 (m, 1H), 3.12 (dd, 1H, J=6.0, 17.0 Hz), 3.33 (dd, 1H, J=5.6, 17.0 Hz), 3.56 (m, 1H), 3,81 (s, 3H), 3.88 (m, 2H), 6.47 (d, 1H, J=7.8 Hz), 7.66 (d, 1H, J=7.8 Hz).

Synthesis of (±)-*trans*-4-Methyl-6-methoxy-9-thiomethoxy-3,4,4a,5,10,10a-hexahydro-2H-naphtho[2,3-b][1,4]oxazine (SDZ NVI 085). To a suspension of CH_3SLi (0.30 g, 5.5 mmol) in 6 ml of DMSO was added 4-methyl-6-methoxy-9-iodo-3,4,4a,5,10,10a-hexahydro-2H-naphtho-[2,3-

b][1,4]oxazine (0.25 g, 0.7 mmol) and Cu_2O (1.3 g, 9.1 mmol). The reaction mixture was stirred for 5 h at 80 °C. It was diluted with EtOAc and washed with 4N NH_4OH several times. The organic layer was dried over Na_2SO_4 and concentrated *in vacuo* to yield an oily residue which was subjected to column chromatography (5% MeOH-CH_2Cl_2) to yield 0.15 g (79%) of the desired product. ^1H NMR (300 MHz, $CDCl_3$): δ 2.09 (m, 1H), 2.32 (dd, 1H, J=10.5, 16.5 Hz), 2.39 (s, 3H), 2.41 (s, 3H), 2.45-2.80 (m, 3H), 3.27-3.40 (m, 2H), 3.60 (m, 1H), 3.82 (s, 3H), 3.90 (m, 2H), 6.72 (d, 1H, J=7.8 Hz), 7.14 (d, 1H, J=7.8 Hz). The product obtained was converted to the HCl salt and recrystallized from EtOAc-Et_2O to obtain 0.17 g of the product as a white solid: mp 215-217 °C; Anal. Cal. For $C_{15}H_{21}NO_2S.1.0HCl$ requires C, 56.9; H, 6.69; N, 4.43. Found: C, 56.5; H, 6.77; N, 4.38.

EXAMPLE 4

Synthesis of 1-(6,7-Dimethoxy-3,4-dihydro-1*H*-isoquinolin-2-yl)ethanone. To a stirred solution of 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (3.00 g, 15.4 mmol, 1.00 equiv) in anhydrous pyridine (100 mL) under argon at room temperature was added acetic anhydride (14.5 mL, 154 mmol, 10.0 equiv) over 15 min. The resulting mixture was stirred at room temperature for 2 h, and then at reflux for 6 h. The volatiles were removed by rotary evaporation at 80 °C under high vacuum. The residue was flash chromatographed on silica gel (MeOH-CH_2Cl_2 8:92) to afford 3.21 g (89%) of viscous brown oil. The ^1H NMR spectrum reflected the presence of two slowly interconverting conformers in a ratio of 1.2 :1 at room temperature. ^1H NMR (300 MHz, $CDCl_3$) for conformer 1: δ 2.18 (s, 3 H), 2.83 (t, J = 5.9 Hz, 2 H), 3.67 (t, J = 5.9 Hz, 2 H), 3.86 (s, 3 H), 3.87 (s, 3 H), 4.66 (s, 2

H), 6.63 (s, 1 H), 6.65 (s, 1 H). For conformer 2: δ 2.19 (s, 3 H), 2.77 (t, J = 5.9 Hz, 2 H), 3.81 (t, J = 5.9 Hz, 2 H), 3.86 (s, 3 H), 3.87 (s, 3 H), 4.56 (s, 2 H), 6.59 (s, 1 H), 6.63 (s, 1 H).

Synthesis of 2-[1-(6,7-Dimethoxy-3,4-dihydro-1*H*-isoquinolin-2-yl)-ethylidineamino]-4,5-dimethoxybenzonitrile. To a stirred solution of 1-(6,7-dimethoxy-3,4-dihydro-1*H*-isoquinolin-2-yl)ethanone (1.00 g, 4.25 mmol, 1.00 equiv) in $CHCl_3$ at room temperature under argon was added $POCl_3$ (143 μL, 1.53 mmol, 0.36 equiv). After 10 min, 2-amino-4,5-dimethoxybenzonitrile (763 mg, 4.28 mmol, 1.01 equiv) was added and the mixture was heated at reflux overnight. The mixture was cooled to room temperature and poured into 1 M aq. NaOH solution (50 mL), and the aqueous phase was extracted with CH_2Cl_2 (3 x 50 mL). The combined organic solutions were dried over $MgSO_4$ and concentrated. The residue was flash chromatographed on silica gel (MeOH-CH_2Cl_2 5:95) to afford 482 mg (28%) of yellow solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.02 (s, 3 H), 2.87 (t, J = 6.0 Hz, 2 H), 3.78 (t, J = 6.0 Hz, 2 H), 3.85 (s, 6 H), 3.87 (s, 6 H), 4.70 (s, 2 H), 6.35 (s, 1 H), 6.65 (s, 2 H), 6.92 (s, 1 H); CIMS (CH_4) 424 $(M + C_2H_5)^+$, 396 $(M + H)^+$.

Synthesis of 2-(6,7-Dimethoxy-3,4-dihydro-1*H*-isoquinolin-2-yl)-6,7-dimethoxyquinolin-4-ylamine hemifumarate hydrate (abanoquil). To a stirred solution of 2-[1-(6,7-dimethoxy-3,4-dihydro-1*H*-isoquinolin-2-yl)-ethylidineamino]-4,5-dimethoxybenzonitrile (471 mg, 1.19 mmol, 1.00 equiv) in refluxing anhydrous *N,N*-dimethylacetamide (24 mL) under argon was added $ZnCl_2$ (339 mg, 2.49 mmol, 2.10 equiv) in three portions over 1 h. The solvent was removed by distillation at 70 °C under high vacuum. Ether (40 mL) was added to the residue,

which was broken up with a stirring rod, and the mixture was stirred at 0°C to precipitate the product. The supernatant was discarded, and the precipitate was washed twice more at 0°C with ether. The solid residue was stirred with 1 M aq. NaOH (25 mL) and CH_2Cl_2 (25 mL) for 10 min, and the aqueous phase was extracted with CH_2Cl_2 (2 x 25 mL). The combined organic solutions were dried over $MgSO_4$ and concentrated to give 493 mg of brown oil, which was flash chromatographed on silica gel (MeOH-CH_2Cl_2 12:88 followed by 2-propylamine-CH_2Cl_2 5:95) to afford 151 mg (38%) of 2-(6,7-dimethoxy-3,4-dihydro-1*H*-isoquinolin-2-yl)-6,7-dimethoxyquinolin-4-ylamine as a tan solid: ^1H NMR (300 MHz, $CDCl_3$) δ 2.90 (t, J = 5.7 Hz, 2 H), 3.81 (t, J = 5.7 Hz, 2 H), 3.86 (s, 3 H), 3.88 (s, 3 H), 3.93 (s, 3 H), 3.97 (s, 3 H), 4.64 (s, 2 H), 6.05 (s, 1 H), 6.66 (s, 1 H), 6.75 (s, 1 H), 7.02 (s, 1 H), 7.23 (s, 1 H); CIMS (CH_4) 424 $(M + C_2H_5)^+$, 396 $(M + H)^+$. To a solution of 2-(6,7-dimethoxy-3,4-dihydro-1*H*-isoquinolin-2-yl)-6,7-dimethoxyquinolin-4-ylamine (150 mg) in hot CH_2Cl_2 (4.5 mL) and MeOH (1.5 mL) was added a solution of fumaric acid (22.8 mg, 0.196 mmol, 0.50 equiv) in hot MeOH (3.0 mL). The resulting mixture was concentrated and the product was recrystallized from MeOH with hot filtration to afford, after filtration, 85 mg of light brown solid: m.p. 239-240 °C. Calcd. for $C_{22}H_{25}N_3O_4 \cdot 0.5\ C_4H_4O_4 \cdot 0.75\ H_2O$: C, 61.73; H, 6.15; N, 9.00. Found: C,61.77; H, 6.17; N, 8.91.

EXAMPLE 5

Synthesis of (±)-2,6-Dimethyl-4-(4-nitrophenyl) -1,4-dihydro-pyridine-3,5-dicarboylicacid[3-(4,4-diphenyl piperidin-1-yl)propyl] ester methyl ester. A solution of methyl 3-aminocrotonate (265 mg, 2.3 mmol, 1.0 equiv), 4-nitrobenzaldehyde (348 mg, 2.3 mmol, 01.0 equiv), and

acetoacetic acid 3-[4,4-diphenylpiperidin-1-yl)propyl] ester (872 mg, 2.3 mmol, 1.0 equiv; Flockerzi, D.; Ulrich, W.-R. U.S. Patent 4,975,440, 1990) in isopropanol was refluxed under argon with stirring for 68 hours. Cooling and removal of solvent gave a residue, which was purified by flash chromatography (SiO_2, EtOAc-hexane 1:1 and 2:1 followed by EtOAc) to afford 717 mg (51%) of yellow solid: ^1H NMR (300 MHz, $CDCl_3$) δ 1.73 (m, 2 H), 2.22 (m, 2 H), 2.30-2.51 (m, 8 H), 2.34 (s, 3 H), 2.35 (s, 3 H), 3.63 (s, 3 H), 4.05 (dt, J = 2.1, 7.9 Hz, 2 H), 5.06 (s, 1 H), 5.73 (br s, 1 H), 7.14 (m, 2 H), 7.27 (m, 8 H), 7.42 (dm, J = 8.8 Hz, 2 H), 8.06 (dm, J = 8.8 Hz, 2 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 15.30, 19.65, 26.32, 36.11, 39.88, 44.60, 50.60, 51.12, 55.34, 62.66, 102.99, 107.55, 123.39, 125.67, 127.12, 128.33, 128.65, 144.80, 144.93, 146.36, 147.50, 154.78, 166.91, 167.43; IR (neat) 1698.0, 1684.7, 1517.5, 1345.7 cm^{-1}; CIMS (NH_3) 610 $(M + 1)^+$, 553, 338.

Synthesis of (±)-2,6-Dimethyl-4-(4-nitrophenyl) -1,4-dihydro-pyridine-3,5-dicarboxylicacid [3-(4,4-diphenylpiperidin-1-yl)propyl] ester methyl ester hydrochloride (Compound 1). To a solution of 2,6-dimethyl-4-(4-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylicacid [3-(4,4-diphenyl-piperidin-1-yl)propyl] ester methyl ester (710 mg, 1.16 mmol, 1.0 equiv) in EtOH (5 mL) was added a solution of HCl in ether (1.0 M, 1.5 mL, 1.5 mmol, 1.3 equiv). The solvents were removed and the residue was dissolved in CH_2Cl_2. This solution was added dropwise to 25 mL of ether to afford, after filtration, 500 mg of yellow crystalline solid: m.p. 152-153 °C. Calcd. for $C_{36}H_{39}N_3O_6 \cdot HCl$: C, 66.92; H, 6.24; N, 6.50. Found: C, 66.70; H, 5.99; N, 6.27.

EXAMPLE 6

Synthesis of (±)-2-Amino-1-(2, 5 dimethoxyphenyl) ethanol (ST-1059)(2, 5, Dimethoxyphenyl)-hydroxy-acetonitrile. To a solution of 4.0 g (24 mmol) of 2,5-dimethoxybenzaldehyde in 40 mL of dichloromethane containing 0.078 g(5% mmol) of KCN and 0.31 g (5% mmol) of 18-crown-6, was added trimethylsilylcyanide 2.62 g(26.4 mmol) dropwise. The reaction mixture was stirred at room temperature for 24 h. The reaction mixture was concentrated, dissolved in chloroform and washed with water, dried (sodium sulfate), concentrated in vacuo and purified by flash chromatography (silica gel; hexane:ethyl acetate, 8:2) to afford 2.85 g (66%) of the desired compound as a yellow oil. ^{1}H NMR (300MHz, $CDCl_3$) δ 6.95 (d, 1H, J = 2.7 Hz), 6.85-6.84 (m, 2H), 5.52 (d, 1H, J = 7.5 Hz), 3.93 (d, 1H, J = 7.5 Hz), 3.83 (s, 3H), 3.73 (s, 3H)

Synthesis of(±)-2-Amino-1-(2, 5 dimethoxyphenyl) ethanol (ST-1059). A solution of 2.84 g (14.7 mmol) of (2, 5 dimethoxyphenyl)-hydroxy acetonitrile in 10 ml of dry THF was stirred and cooled using ice bath as a 1 M solution of borane in THF (90 mL) was added dropwise. After the addition was complete, the mixture was heated at reflux for 20 h. It was cooled and treated with 40 mL of 6 N hydrochloric acid and washed with ethyl acetate.The aqueous layer was neutralized with 1 N sodium hydroxide and extracted with ethyl acetate and concentrated to afford the desired compound as a white solid 1.5 g (52%). ^{1}H NMR (300MHz, $CDCl_3$) δ 6.97 (d, 1H, J = 2.5 Hz), 6.73-6.69(m, 2H), 4.83-4.81(t, 1H, J = 4.1 Hz), 3.72(s, 3H), 3.71(s, 3H), 2.89-2.65(m, 2H), 2.16(brs, 2H)

EXAMPLE 7

The binding and functional properties of compounds at the different human receptors were determined *in vitro* using cultured cell lines that selectively express the receptor of interest. These cell lines were prepared by transfecting the cloned cDNA or cloned genomic DNA or constructs containing both genomic DNA and cDNA encoding the human α-adrenoceptors as follows:

Human α_{1A} Adrenoceptor. The entire coding region of the α1A receptor (1719 bp), including 150 basepairs of 5′ untranslated sequence (5′ UT) and 300 bp of 3′ untranslated sequence (3′ UT), was cloned into the BamHI and ClaI sites of the polylinker-modified eukaryotic expression vector pCEXV-3, called EXJ.HR. The construct involved the ligation of partial overlapping human lymphocyte genomic and hippocampal cDNA clones: 5′ sequences were contained on a 1.2 kb SmaI-XhoI genomic fragment (the vector-derived BamHI site was used for subcloning instead of the internal insert-derived SmaI site) and 3′ sequences were contained on an 1.3 kb XhoI-ClaI cDNA fragment (the ClaI site was from the vector polylinker). Stable cell lines were obtained by cotransfection with the plasmid α1A/EXJ (expression vector containing the α1A receptor gene) and the plasmid pGCcos3neo (plasmid containing the aminoglycoside transferase gene) into LM(tk$^-$), CHO, and NIH3T3 cells, using calcium phosphate technique. The cells were grown, in a controlled environment (37°C., 5% CO_2), as monolayers in Dulbecco's modified Eagle's Medium (GIBCO, Grand Island, NY) containing 25mM glucose and supplemented with 10% bovine calf serum, 100 units/ml penicillin g, and 100 μg/ml streptomycin sulfate. Stable clones were then selected for resistance to the antibiotic G-418 (1

mg/ml), and membranes were harvested and assayed for their ability to bind [^{3}H]prazosin as described below (see "Radioligand Binding assays").

Human α_{1B} Adrenoceptor. The entire coding region of the α1B receptor (1563 bp), including 200 basepairs and 5' untranslated sequence (5' UT) and 600 bp of 3' untranslated sequence (3' UT), was cloned into the EcoRI site of pCEXV-3 eukaryotic expression vector. The construct involved ligating the full-length containing EcoRI brainstem cDNA fragment from λ ZapII into the expression vector. Stable cell lines were obtained as described above.

Human α_{1C} Adrenoceptor. The entire coding region of the α1C receptor (1401 bp), including 400 basepairs of 5' untranslated sequence (5' UT) and 200 bp of 3' untranslated sequence (3' UT), was cloned into the KpnI site of the polylinker-modified pCEXV-3-derived eukaryotic expression vector, EXJ.RH. The construct involved ligating three partial overlapping fragments: a 5' 0.6kb HincII genomic clone, a central 1.8 EcoRI hippocampal cDNA clone, and a 3' 0.6Kb PstI genomic clone. The hippocampal cDNA fragment overlaps with the 5' and 3' genomic clones so that the HincII and PstI sites at the 5' and 3' ends of the cDNA clone, respectively, were utilized for ligation. This full-length clone was cloned into the KpnI site of the expression vector, using the 5' and 3' KpnI sites of the fragment, derived from vector (i.e., pBluescript) and 3'-untranslated sequences, respectively. Stable cell lines were obtained as described above.

Radioligand Binding Assays.

Human α_1-Adrenoceptors. Transfected cells from culture flasks were scraped into 5ml of 5mM Tris-HCl, 5mM EDTA, pH 7.5, and lysed by sonication. The cell lysates were centrifuged at 1000 rpm for 5 min at 4°C, and the supernatant was centrifuged at 30,000 x g for 20 min at 4°C. The pellet was suspended in 50mM Tris-HCl, 1mM $MgCl_2$, and 0.1% ascorbic acid at pH 7.5. Binding of the α1 antagonist [^{3}H]prazosin (0.5 nM, specific activity: about 76.2 Ci/mmol) to membrane preparations of LM(tk-) cells was done in a final volume of 0.25 ml and incubated at 37°C for 20 min. Nonspecific binding was defined as that binding which remained in the presence of 10 μM phentolamine(a concentration at least 100-fold greater than the affinity of phentolamine at any human α-adrenoceptors). The reaction was stopped by filtration through GF/B filters using a cell harvester. Equilibrium competition binding assays, routinely consisting of 7 different concentrations of the tested compounds, were analyzed using a non-linear regression curve-fitting computer program to obtain IC_{50} values. The IC_{50} values were converted to affinity constants (pK_I) by the method of Cheng and Prusoff (1973).

Human α_2-Adrenoceptors. To determine the affinity of compounds at the α_2 receptors, LM(tk-) cell lines stably transfected with the genes encoding the α_{2A}, α_{2B}, and α_{2C} receptors were used. Cell lysates were prepared as described above (see Radioligand Binding Assays), and suspended in 25mM glycylglycine buffer (pH 7.6 at room temperature). Equilibrium competition binding assays were performed using [^{3}H]rauwolscine (0.5nM), and nonspecific binding was determined by incubation with 10μM phentolamine. The bound radioligand was separated

by filtration through GF/B filters using a cell harvester.

Human Histamine H_1 Receptor. The coding sequence of the human histamine H_1 receptor, homologous to the bovine H_1 receptor, was obtained from a human hippocampal cDNA library, and was cloned into the eukaryotic expression vector pCEXV-3. The plasmid DNA for the H_1 receptor is designated pcEXV-H1, and was deposited on November 6, 1992 under ATCC Accession No. 75346. This construct was transfected into COS-7 cells by the DEAE-dextran method. Cells were harvested after 72 hours and lysed by sonication in 5mM Tris-HCl, 5mM EDTA, pH 7.5. The cell lysates were centrifuged at 1000 rpm for 5 min at 4°C, and the supernatant was centrifuged at 30,000 x g for 20 min. at 4°C. The pellet was suspended in 37.8 mM $NaHPO_4$, 12.2 mM KH_2PO_4, pH 7.5. The binding of the histamine H_1 antagonist [^{3}H]mepyramine (1nM, specific activity: about 24.8 Ci/mM) was done in a final volume of 0.25 ml and incubated at room temperature for 60 min. Nonspecific binding was determined in the presence of 10 μM mepyramine. The bound radioligand was separated by filtration through GF/B filters using a cell harvester.

Human Histamine H_2 Receptor. The coding sequence of the human H_2 receptor was obtained from a human placenta genomic library, and cloned into the cloning site of PCEXV-3 eukaryotic expression vector. The plasmid DNA for the H_2 receptor is designated pcEXV-H2, and was deposited on November 6, 1992 under ATCC Accession No. 75345. This construct was transfected into COS-7 cells by the DEAE-dextran method. Cells were harvested after 72 hours and lysed by sonication in 5mM Tris-HCl, 5mM EDTA, pH 7.5. The cell lysates were centrifuged at 1000 rpm for 5 min at 4°C, and the supernatant was centrifuged

at 30,000 x g for 20 min at 4 °C. The pellet was suspended in 37.8 mM $NaHPO_4$, 12.2 mM $K2PO_4$, pH 7.5. The binding of the histamine H_2 antagonist [^{3}H]tiotidine (5nM, specific activity: about 70 Ci/mM) was done in a final volume of 0.25 ml and incubated at room temperature for 60 min. Nonspecific binding was determined in the presence of 10 μM histamine. The bound radioligand was separated by filtration through GF/B filters using a cell harvester.

Human Serotonin Receptors. 5-$HT_{1D\alpha}$, 5-$HT_{1D\beta}$, 5-HT_{1E}, 5-HT_{1F}, and 5-HT_7 Receptors: The cell lysates of LM(tk-) clonal cell line stably transfected with the genes encoding each of these 5-HT receptor-subtypes were prepared as described above. The cell line for the 5-$HT_{1D\alpha}$ receptor, designated as Ltk-8-30-84, was deposited on April 17, 1990, and accorded ATCC Accession No. CRL 10421. The cell for the 5-$HT_{1D\beta}$ receptor, designated as Ltk-11, was deposited on April 17, 1990, and accorded ATCC Accession No. CRL 10422. The cell line for the 5-HT_{1E} receptor, designated 5-HT_{1E}-7, was deposited on November 6, 1991, and accorded ATCC Accession No. CRL 10913. The cell line for the 5-HT_{1F} receptor, designated L-5-HT_{1F}, was deposited on December 27, 1991, and accorded ATCC Accession No. CRL 10957. The cell line for the 5-HT_7 receptor, designated as L-5-HT-4B, was deposited on October 20, 1992, and accorded ATCC Accession No. CRL 11166. These preparations were suspended in 50mM Tris-HCl buffer (pH 7.4 at 37°C) containing 10 mM $MgCl_2$, 0.2 mM EDTA, 10μM pargyline, and 0.1% ascorbate. The affinities of compounds were determined in equilibrium competition binding assays by incubation for 30 minutes at 37°C in the presence of 5nM [^{3}H]serotonin. Nonspecific binding was determined in the presence of 10μM serotonin. The bound radioligand was separated by filtration through

GF/B filters using a cell harvester.

Human 5-HT_2 Receptor. The coding sequence of the human 5-HT_2 receptor was obtained from a human brain cortex cDNA library, and cloned into the cloning site of pCEXV-3 eukaryotic expression vector. This construct was transfected into COS-7 cells by the DEAE-dextran method. Cells were harvested after 72 hours and lysed by sonication in 5mM Tris-HCl, 5mM EDTA, pH 7.5. This cell line was deposited with the ATCC on October 31, 1989, designated as L-NGC-5-HT_2, and was accorded ATCC Accession No. CRL 10287. The cell lysates were centrifuged at 1000 rpm for 5 minutes at 4°C, and the supernatant was centrifuged at 30,000 x g for 20 minutes at 4°C. The pellet was suspended in 50mM Tris-HCl buffer (pH 7.7 at room temperature) containing 10 mM $MgSO_4$, 0.5mM EDTA, and 0.1% ascorbate. The affinity of compounds at 5-HT_2 receptors were determined in equilibrium competition binding assays using [^{3}H]ketanserin (1nM). Nonspecific binding was defined by the addition of 10μM mianserin. The bound radioligand was separated by filtration through GF/B filters using a cell harvester.

5-HT_{1A} receptor. The cell line for the 5-HT_{1A} receptor, designated 5-HT1A-3, was deposited on May 11, 1995, and accorded ATCC Accession No. CRL 11889. The cDNA corresponding to the 5-HT_{1A} receptor open reading frames and variable non-coding 5'- and 3'-regions, was cloned into the eukaryotic expression vector pCEXV-3. These constructs were transfected transiently into COS-7 cells by the DEAE-dextran method, and harvested after 72 hours. Radioligand binding assays were performed as described above for the 5-HT_2 receptor, except that [^{3}H]-8-OH-DPAT was used as the radioligand and nonspecific binding was

determined by the addition of 10μM mianserin.

Human Dopamine D_2 Receptors. The affinity of compounds at the D2 receptor were determined using membrane preparations from COS-7 cells transfected with the gene encoding the human D_2 receptor. The coding region for the human D2 receptor was obtained from a human striatum cDNA library, and cloned into the cloning site of PCDNA 1 eukariotic expression vector. The plasmid DNA for the D_2 receptor is designated pcEXV-D2, and was deposited on November 6, 1992 under ATCC Accession No. 75344. This construct was transfected into COS-7 cells by the DEAE-dextran method. Cells were harvested after 72 hours and lysed by sonication in 5mM Tris-HCl, 5mM EDTA, pH 7.5. The cell lysates were centrifuged at 1000 rpm for 5 minutes at 4°C, and the supernatant was centrifuged at 30,000 x g for 20 minutes at 4°C. The pellet was suspended in 50 mM Tris-HCl (pH 7.4) containing 1mM EDTA, 5mM KCl, 1.5mM $CaCl_2$, 4mM $MgCl_2$, and 0.1% ascorbic acid. The cell lysates were incubated with [^{3}H]spiperone (2nM), using 10μM (+)Butaclamol to determine nonspecific binding.

Other Dopamine receptors were prepared by known methods. (D_1: Dearry et al., Nature, 347, 72, (1990), deposited with the European Molecular Biological Laboratory (EMBL) Genbank as X55760; D_3: Sokoloff, P. et al., Nature, 347, 146 (1990), deposited with the European Molecular Biological Laboratory (EMBL) Genbank as X53944; D_5: Sunahara, R.K., et al., Nature, 350, 614 (1991), deposited with EMBL Genbank as X58454-HU HD 5DR).

Functional Assays.

α_1-Adrenoceptor-Mediated Phosphoinositide Accumulation in Cultured Cell Lines. The agonist activity of test compounds was assayed by measuring their ability to generate phosphoinositide production in cells stably transfected with each of the three cloned human α_1-adrenoceptor subtypes. Cells were plated in 96-well plates and grown to confluence. The day before the assay the growth medium was changed to 100 µl of medium containing 1% serum and 0.5 µCi [^{3}H]*myo*-inositol, and the plates were incubated overnight in a CO_2 incubator (5% CO_2 at 37°C). Immediately before the assay, the medium was removed and replaced by 200 µl of PBS containing 10 mM LiCl, and the cells were equilibrated with the new medium for 20 min. During this interval cells were also equilibrated with the antagonist, added as 10 µl aliquot of a 20-fold concentrated solution in PBS.

The [^{3}H]inositol-phosphate (IP) accumulation was started by adding 10 µl of a solution containing the agonist. To the first well 10 µl were added to measure basal accumulation, and 11 different concentrations of agonist were assayed in the following 11 wells of each plate row. All assays were performed in duplicate by repeating the same additions in two consecutive plate rows. The plates were incubated in a CO_2 incubator for 1 hr. The reaction was terminated by adding 15 µl of 50% (v/v) trichloroacetic acid (TCA), followed by a 40 min incubation at 4°C.

After neutralizing TCA with 40µl of 1M Tris, the content of the wells was transferred to a Multiscreen HV filter plate (Millipore) containing Dowex AG1-X8 (200-400 mesh, formate form). The filter plates were prepared adding 200

μl of Dowex AG1-X8 suspension (50% v/v, water:resin) to each well. The filter plates were placed on a vacuum manifold to wash or elute the resin bed. Each well was washed 2 times with 200 μl of water, followed by 2 x 200 μl of 5mM sodium tetraborate/60 mM ammonium formate. The [^{3}H] IPs were eluted into empty 96-well plates with 200 μl of 1.2 M ammonium formate/0.1 formic acid. The content of the wells was added to 3 mls of scintillation cocktail, and the radioactivity was determined by liquid scintillation counting.

<u>α_2-Adrenoceptor-Mediated Inhibition of Forskolin-Stimulated Adenylyl Cyclase.</u> The agonist activity of test compounds was assayed by measuring their ability to inhibit adenylyl cyclase in cells stably transfected with each of the three cloned human α_2-adrenoceptors. LM(tk-) cells expressing the α_{2A}- or the α_{2C}-, as well as Y1 cells expressing the α_{2B}-adrenoceptor were used. The cell line for the α_{2B}-adrenoceptor, designated as Ya2B-2, was deposited on May 11, 1995, and accorded ATCC Accession No. CRL 11888. The formation of cyclic AMP was measured in cultures incubated with DMEM containing 1 mM theophylline. Twelve concentrations of the test compounds (from 10 pM to 100μM) were added to the incubation medium and incubated at 37°C for 20 min. Following this incubation step, 10 μM forskolin was added to stimulate the formation of cyclic AMP, and the cultures were incubated for another 10 min. The reaction was stopped by replacing the incubation medium with 100 mM HCl. The intracellular levels of cyclic AMP were measured by radioimmunoassay. The data from concentration-response curves was fitted to a four-parameter logistic equation, by non-linear regression analysis, to determine the pEC_{50} and intrinsic activity.

Isolated Tissue Assays.

Protocol for the Identification of α_1-Adrenoceptors in Mammalian Urethra from Functional Studies.

Using a battery of agonists and antagonists which exhibit selectivity among the α_1-adrenoceptor subtypes, a pharmacological profile of the receptor which mediates the contractile response to α-agonists in the urethra of male humans, and male and female dogs and rabbits was determined. In addition, similar studies were done using bladder neck tissue from female dogs. In order to identify the specific receptor subtype in each tissue, the pharmacological profile was compared to the profiles for these same drugs at the cloned human α_{1A}, α_{1B}, or α_{1C} subtypes.

Methods.

Tissue samples from the proximal urethra of male humans and male and female rabbits, as well as tissue samples from both the proximal urethra and bladder neck of male and female dogs were cut into transverse strips (3x10mm) and suspended under 0.5g tension in Krebs' physiological buffer at 37°C. To determine agonist potency (pEC_{50}) and antagonist affinity constants (pK_B), concentration-effect curves to the non-selective agonist phenylepherine were constructed in the absence and in the presence of increasing concentrations of the antagonist. Up to four sequential curves were constructed in each tissue. Antagonists were allowed to equilibrate for 1h before each concentration-effect curve, and the drugs were completely washed out in between successive curves. In each experiment, one tissue served as a "time control", in which no antagonist was added so that changes in

tissue sensitivity could be assessed as a function of time. In most instances, antagonist pK_B values were determined by Schild analysis (Arunlakshana and Schild,1959). In instances in which a single concentration of antagonist was used, pK_B values were determined by the equation: pK_B = log ((CR-1)/[Antagonist]), where CR is defined as the ratio of the agonist EC_{50} in the presence of the antagonist to that in the absence of the antagonist. In addition to the antagonist studies, the α_{1C}-selective agonists A-61603 and SK&F 102652 were used to characterize the receptor subtype in the female dog urethra, and to compare the receptor profiles in the urethra with that in the bladder neck. In these experiments, two concentration effect curves were constructed on each tissue, one in the absence and the second in the presence of prazosin. The pK_B values derived for prazosin using A-61603 and SK&F 102652 as the agonists were compared to the pK_B obtained for prazosin using phenylephrine as the agonist, to verify that each agonist interacted with a common α_1-adrenoceptor site.

Determination of β-Adrenoceptor Activity in the Isolated Rat Right Atrium. Right atria were removed from rats and placed immediately into oxygenated Krebs solution at 37°C. The Krebs solution was replaced three times at 5 min intervals and the tissues were tensioned three times to 0.5g. In spontaneously beating atria, a control concentration effect curve to isoprenaline was generated. β-Adrenoceptor-mediated increase in atrial rate were measured as the response. After complete wash out of the isoprenaline, a concentration effect curve was performed using the agonists A-61603, SK&F 102652, and SDZ NVI 085, up to a concentration of 100μM. If no response was observed, the drug was left in the bath while another

concentration-effect curve to isoprenaline was generated. pEC_{50} values for agonists were calculated by logistic curve fitting. The antagonist effects of the test compounds were measured using the dose-ratio method, by comparing the shift in pEC_{50} for isoprenaline.

Results.

Table 2 shows the pK_I values determined from binding assays for various antagonists at the cloned human α_1-adrenoceptor subtypes and the corresponding pK_B values determined from contractile studies in urethral and bladder neck tissues obtained from human, dog, and rabbit.

Figures 1-5 illustrate the data from Table 2 in a graphical format. For each of the mammalian tissues, the pK_B value for each antagonist (abcissa) is plotted against the pK_I values determined for each of the three cloned human α-adrenoceptor subtypes (ordinate). The slopes and correlation coefficients (r) for the linear regression analysis are presented in each figure. In each case, the antagonist data derived from the functional experiments correlates best with the α_{1C}-subtype.

Table 3 shows the pK_I, pEC_{50}, and intrinsic activity at the cloned α_1- and α_2-subtypes for the various agonists. In particular, A-61603 and SK&F 102652 each fully stimulate inositol phosphate production in cells transfected with the human α_{1C}-adrenoceptor, but are virtually inactive at the α_{1A}- and α_{1B}-subtypes. Table 3 also indicates that while both A-61603 and SK&F 102652 are selective among the α_1-adrenoceptors, these compounds also possess significant activity at α_2-adrenoceptors. The cross-reactivity binding profiles of these drugs and

other agonists are shown in Table 4.

Because of the ability of A-61603 and SK&F 102652 to fully stimulate α_{1C}-adrenoceptors, but not α_{1A}- or α_{1B}-subtypes, these compounds were used in the female dog tissues to compliment the antagonist-based pharmacological characterization. Additionally, these two compounds were used to establish that the α_1-subtype in the urethra is identical to the α_1-subtype in the bladder neck. The potency of these agonists and the pK_B value for prazosin in antagonizing their effects in each tissue are as follows:

Bladder Neck	pEC_{50}	Prazosin pK_B
A-61603	6.8	8.3
SK&F 102652	5.7	7.8

Urethra	pEC_{50}	Prazosin pK_B
A-61603	6.7	8.3
SK&F 102652	6.0	8.5

In each tissue, the magnitude of the contractions produced by A-61603 and SK&F 102652 was similar to the magnitude of the contraction produced by phenylephrine. In addition, the contractions produced by A-61603 and SK&F 102652 were highly sensitive to prazosin, confirming their action at an α_1-adrenoceptor site. The high degree of selectivity of these compounds for the α_{1C}-subtype over the α_{1A} and α_{1B} subtypes, indicates that it is the α_{1C}-subtype which mediates contraction of the urethra as well as the bladder neck.

Table 2. Antagonist affinities (pK_I versus ^{3}H-prazosin binding) at human cloned α_1-adrenoceptors and antagonist affinities determined from contractile studies (pK_B versus phenylephrine-induced contraction) in urethra and bladder neck (BN) tissue from mammalian species. (* 5-methyl urapidil)

Antagonist	Human Clones			Human Urethra Male	Rabbit Urethra Male	Rabbit Urethra Female	Dog BN Male	Dog BN Female
	$h\alpha_{1A}$	$h\alpha_{1B}$	$h\alpha_{1C}$					
Prazosin	9.5	9.3	9.2	9.5	7.8	7.8	8.2	8.0
Abanoquil	10.4	10.1	10.4	9.6	8.7	8.7	8.6	--
5-MU *	7.8	6.9	8.7	8.8	7.8	7.4	8.7	8.8
BMY 7378	9.0	7.0	6.8	7.5	6.5	6.1	7.1	6.8
Compound 1	6.5	7.1	8.5	8.1	--	--	6.2	--

Table 3. Pharmacological profiles of agonists at cloned human α-adrenoceptors. Binding affinity (pKi), potency (pEC_{50}), and intrinsic activity were determined as described in the in the text.

Compound		α_{1A}	α_{1B}	α_{1C}	α_{2A}	α_{2B}	α_{2C}
(-)-ephedrine	pKi	4.3	4.0	4.6	6.1	5.6	5.1
	pEC_{50}	4.0	4.0	4.0	6.6	6.6	5.0
	i.a.	0.1	0.2	0.1	0.3	0.4	0.0
(-)-norephedrine	pKi	4.6	4.3	4.8	6.3	6.6	5.5
	pEC_{50}	4.8	4.5	4.3	8.1	6.1	6.9
	i.a.	0.3	0.0	0.4	0.5	0.5	0.6
(-)-phenylephrine	pKi	5.4	4.7	4.1	6.9	6.4	6.4
	pEC_{50}	5.9	5.7	5.9	6.9	6.9	6.4
	i.a.	0.7	0.9	0.9	1.0	0.8	0.9
ST-1059	pKi	5.1	4.9	5.1	5.7	5.8	5.4
	pEC_{50}	5.8	5.2	5.7	5.6	8.6	6.3
	i.a.	0.1	0.1	0.9	0.2	0.6	0.5
A-61603	pKi	4.9	4.8	7.1	7.3	6.5	6.2
	pEC_{50}	4.6	4.4	8.9	7.5	7.1	7.7
	i.a.	0.1	0.1	1.2	0.8	0.8	0.9
SK&F 102652	pKi	4.9	4.4	5.6	6.4	6.5	5.8
	pEC_{50}	4.7	4.3	6.9	<4.0	7.5	7.1
	i.a.	0.1	0.1	1.1	0.0	0.9	0.8
SDZ NVI-085	pKi	5.7	4.9	5.9	7.3	7.2	6.1
	pEC_{50}	<4.0	<4.0	6.4	<4.0	<4.0	<4.0
	i.a.	0.0	0.0	1.1	0.0	0.0	0.0

Table 4. Cross-reactivity receptor binding profiles at human cloned histamine subtypes (H1, H2), dopamine subtypes (D1, D2, D3, D5), and serotonin subtypes (5-HT: 1A, 1Dα, 1Dβ, 1E, 1F, 2, 7), as well as rat atrial β-adrenoceptors. Affinities (pKi) were determined as described in the text.

Compound	H1	H2	D1	D2	D3	D5	1A	1Dα	1Dβ	1E	1F	2	7	β
A-61603	4.3	4.8	4.7	5.0	5.8	4.8	5.4	5.6	5.2	5.3	5.3	4.3	5.3	<4
SK&F 102652	4.3	4.6	5.0	6.7	6.7	4.8	5.8	5.9	5.5	5.3	5.3	4.8	5.3	<4
SDZ NVI-085	5.3	5.2	5.1	5.5	6.0	4.7	7.1	7.9	7.7	5.7	7.0	6.5	7.0	<4

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What is claimed is:

1. A method of treating urinary incontinence in a subject which comprises administering to the subject a therapeutically effective amount of an α_{1C} selective agonist which activates a human α_{1C} adrenoceptor at least ten-fold more than it activates a human α_{1A} adrenoceptor and a human α_{1B} adrenoceptor.

2. The method of claim 1, wherein the α_{1C} selective agonist further has the characteristic that it does not antagonize a human α_{1A} adrenoceptor and a human α_{1B} adrenoceptor.

3. The method of claim 1, wherein the α_{1C} selective agonist activates the human α_{1C} adrenoceptor at least ten-fold more than it activates any human α_2 adrenoceptor and any β adrenoceptor.

4. The method of claim 1, wherein the α_{1C} selective agonist further has the characteristic that it does not antagonize any human α_2 adrenoceptor and any β adrenoceptor.

5. The method of claim 1, wherein the α_{1C} selective agonist activates the human α_{1C} adrenoceptor at least ten-fold more than it activates a human histamine H_1 or H_2 receptor.

6. The method of claim 1, wherein the α_{1C} selective agonist activates the human α_{1C} adrenoceptor at least ten-fold more than it activates a human dopamine D_1, D_2, D_3, or D_5 receptor.

7. The method of claim 1, wherein the α_{1C} selective agonist activates the human α_{1C} adrenoceptor at

least ten-fold more than it activates a human serotonin 5-HT_{1A}, 5-$HT_{1D\alpha}$, 5-$HT_{1D\beta}$, 5-HT_{1E}, 5-HT_{1F}, 5-HT_2, or 5-HT, receptor.

8. A method of treating urinary incontinence in a subject which comprises administering to the subject a therapeutically effective amount of an α_{1C} selective agonist which activates a human α_{1C} adrenoceptor at least 50-fold more than it activates a human α_{1A} adrenoceptor and a human α_{1B} adrenoceptor.

9. The method of claim 8, wherein the α_{1C} selective agonist further has the characteristic that it does not antagonize a human α_{1A} adrenoceptor and a human α_{1B} adrenoceptor.

10. The method of claim 8, wherein the α_{1C} selective agonist activates the human α_{1C} adrenoceptor at least ten-fold more than it activates any human α_2 adrenoceptor and any β adrenoceptor.

11. The method of claim 8, wherein the α_{1C} selective agonist further has the characteristic that it does not antagonize any human α_2 adrenoceptor and any β adrenoceptor.

12. The method of claim 8, wherein the α_{1C} selective agonist activates the human α_{1C} adrenoceptor at least ten-fold more than it activates a human histamine H_1 or H_2 receptor.

13. The method of claim 8, wherein the α_{1C} selective agonist activates the human α_{1C} adrenoceptor at least ten-fold more than it activates a human dopamine D_1, D_2, D_3, or D_5 receptor.

14. The method of claim 8, wherein the α_{1C} selective agonist activates the human α_{1C} adrenoceptor at least ten-fold more than it activates a human serotonin 5-HT_{1A}, 5-$HT_{1D\alpha}$, 5-$HT_{1D\beta}$, 5-HT_{1E}, 5-HT_{1F}, 5-HT_2, or 5-HT_7 receptor.

15. A method of treating urinary incontinence in a subject which comprises administering to the subject a therapeutically effective amount of an α_{1C} selective agonist which activates a human α_{1C} adrenoceptor at least 100-fold more than it activates a human α_{1A} adrenoceptor and a human α_{1B} adrenoceptor.

16. The method of claim 15, wherein the α_{1C} selective agonist further has the characteristic that it does not antagonize a human α_{1A} adrenoceptor and a human α_{1B} adrenoceptor.

17. The method of claim 15, wherein the α_{1C} selective agonist activates the human α_{1C} adrenoceptor at least ten-fold more than it activates any human α_2 adrenoceptor and any β adrenoceptor.

18. The method of claim 15, wherein the α_{1C} selective agonist further has the characteristic that it does not antagonize any human α_2 adrenoceptor and any β adrenoceptor.

19. The method of claim 15, wherein the α_{1C} selective agonist activates the human α_{1C} adrenoceptor at least ten-fold more than it activates a human histamine H_1 or H_2 receptor.

20. The method of claim 15, wherein the α_{1C} selective agonist activates the human α_{1C} adrenoceptor at least ten-fold more than it activates a human

dopamine D_1, D_2, D_3, or D_5 receptor.

21. The method of claim 15, wherein the α_{1C} selective agonist activates the human α_{1C} adrenoceptor at least ten-fold more than it activates a human serotonin 5-HT_{1A}, 5-$HT_{1D\alpha}$, 5-$HT_{1D\beta}$, 5-HT_{1E}, 5-HT_{1F}, 5-HT_2, or 5-HT_7 receptor.

22. A method of treating urinary incontinence in a subject which comprises administering to the subject a therapeutically effective amount of an α_{1C} selective agonist which activates a human α_{1C} adrenoceptor at least 200-fold more than it activates a human α_{1A} adrenoceptor and a human α_{1B} adrenoceptor.

23. The method of claim 22, wherein the α_{1C} selective agonist further has the characteristic that it does not antagonize a human α_{1A} adrenoceptor and a human α_{1B} adrenoceptor.

24. The method of claim 22, wherein the α_{1C} selective agonist activates the human α_{1C} adrenoceptor at least ten-fold more than it activates any human α_2 adrenoceptor and any β adrenoceptor.

25. The method of claim 22, wherein the α_{1C} selective agonist further has the characteristic that it does not antagonize any human α_2 adrenoceptor and any β adrenoceptor.

26. The method of claim 22, wherein the α_{1C} selective agonist activates the human α_{1C} adrenoceptor at least ten-fold more than it activates a human histamine H_1 or H_2 receptor.

27. The method of claim 22, wherein the α_{1C} selective

agonist activates the human α_{1C} adrenoceptor at least ten-fold more than it activates a human dopamine D_1, D_2, D_3, or D_5 receptor.

28. The method of claim 22, wherein the α_{1C} selective agonist activates the human α_{1C} adrenoceptor at least ten-fold more than it activates a human serotonin 5-HT_{1A}, 5-$HT_{1D\alpha}$, 5-$HT_{1D\beta}$, 5-HT_{1E}, 5-HT_{1F}, 5-HT_2, or 5-HT_7 receptor.

29. A method of inducing contraction of urethra and bladder neck tissues which comprises contacting the urethra and bladder neck tissues with an effective contraction-inducing amount of an α_{1C} selective agonist which activates a human α_{1C} adrenoceptor at least ten-fold more than it activates a human α_{1A} adrenoceptor and a human α_{1B} adrenoceptor.

30. The method of claim 29, wherein the α_{1C} selective agonist further has the characteristic that it does not antagonize a human α_{1A} adrenoceptor and a human α_{1B} adrenoceptor.

31. The method of claim 29, wherein the α_{1C} selective agonist activates the human α_{1C} adrenoceptor at least ten-fold more than it activates any human α_2 adrenoceptor and any β adrenoceptor.

32. The method of claim 29, wherein the α_{1C} selective agonist further has the characteristic that it does not antagonize any human α_2 adrenoceptor and any β adrenoceptor.

33. The method of claim 29, wherein the α_{1C} selective agonist activates the human α_{1C} adrenoceptor at least ten-fold more than it activates a human

histamine H_1 or H_2 receptor.

34. The method of claim 29, wherein the α_{1C} selective agonist activates the human α_{1C} adrenoceptor at least ten-fold more than it activates a human dopamine D_1, D_2, D_3, or D_5 receptor.

35. The method of claim 29, wherein the α_{1C} selective agonist activates the human α_{1C} adrenoceptor at least ten-fold more than it activates a human serotonin 5-HT_{1A}, 5-$HT_{1D\alpha}$, 5-$HT_{1D\beta}$, 5-HT_{1E}, 5-HT_{1F}, 5-HT_2, or 5-HT, receptor.

36. A method of inducing contraction of urethra and bladder neck tissues which comprises contacting the urethra and bladder neck tissues with an effective contraction-inducing amount of an α_{1C} selective agonist which activates a human α_{1C} adrenoceptor at least 50-fold more than it activates a human α_{1A} adrenoceptor and a human α_{1B} adrenoceptor.

37. A method of claim 36, wherein the α_{1C} selective agonist further has the characteristic that it does not antagonize a human α_{1A} adrenoceptor and a human α_{1B} adrenoceptor.

38. The method of claim 36, wherein the α_{1C} selective agonist activates the human α_{1C} adrenoceptor at least ten-fold more than it activates any human α_2 adrenoceptor and any β adrenoceptor.

39. The method of claim 36, wherein the α_{1C} selective agonist further has the characteristic that it does not antagonize any human α_2 adrenoceptor and any β adrenoceptor.

40. The method of claim 36, wherein the α_{1C} selective agonist activates the human α_{1C} adrenoceptor at least ten-fold more than it activates a human histamine H_1 or H_2 receptor.

41. The method of claim 36, wherein the α_{1C} selective agonist activates the human α_{1C} adrenoceptor at least ten-fold more than it activates a human dopamine D_1, D_2, D_3, or D_5 receptor.

42. The method of claim 36, wherein the α_{1C} selective agonist activates the human α_{1C} adrenoceptor at least ten-fold more than it activates a human serotonin 5-HT_{1A}, 5-$HT_{1D\alpha}$, 5-$HT_{1D\beta}$, 5-HT_{1E}, 5-HT_{1F}, 5-HT_2, or 5-HT_7 receptor.

43. A method of inducing contraction of urethra and bladder neck tissues which comprises contacting the urethra and bladder neck tissues with an effective contraction-inducing amount of an α_{1C} selective agonist which activates a human α_{1C} adrenoceptor at least 100-fold more than it activates a human α_{1A} adrenoceptor and a human α_{1B} adrenoceptor.

44. The method of claim 43, wherein the α_{1C} selective agonist further has the characteristic that it does not antagonize a human α_{1A} adrenoceptor and a human α_{1B} adrenoceptor.

45. The method of claim 43, wherein the α_{1C} selective agonist activates the human α_{1C} adrenoceptor at least ten-fold more than it activates any human α_2 adrenoceptor and any β adrenoceptor.

46. The method of claim 43, wherein the α_{1C} selective agonist further has the characteristic that it

does not antagonize any human α_2 adrenoceptor and any β adrenoceptor.

47. The method of claim 43, wherein the α_{1C} selective agonist activates the human α_{1C} adrenoceptor at least ten-fold more than it activates a human histamine H_1 or H_2 receptor.

48. The method of claim 43, wherein the α_{1C} selective agonist activates the human α_{1C} adrenoceptor at least ten-fold more than it activates a human dopamine D_1, D_2, D_3, or D_5 receptor.

49. The method of claim 43, wherein the α_{1C} selective agonist activates the human α_{1C} adrenoceptor at least ten-fold more than it activates a human serotonin 5-HT_{1A}, 5-$HT_{1D\alpha}$, 5-$HT_{1D\beta}$, 5-HT_{1E}, 5-HT_{1F}, 5-HT_2, or 5-HT_7 receptor.

50. A method of inducing contraction of urethra and bladder neck tissues which comprises contacting the urethra and bladder neck tissues with an effective contraction-inducing amount of an α_{1C} selective agonist which activates a human α_{1C} adrenoceptor at least 200-fold more than it activates a human α_{1A} adrenoceptor and a human α_{1B} adrenoceptor.

51. The method of claim 50, wherein the α_{1C} selective agonist further has the characteristic that it does not antagonize a human α_{1A} adrenoceptor and a human α_{1B} adrenoceptor.

52. The method of claim 50, wherein the α_{1C} selective agonist activates the human α_{1C} adrenoceptor at least ten-fold more than it activates any human α_2 adrenoceptor and any β adrenoceptor.

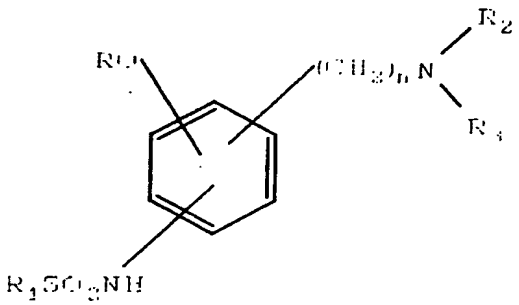
53. The method of claim 50, wherein the α_{1C} selective agonist further has the characteristic that it does not antagonize any human α_2 adrenoceptor and any β adrenoceptor.

54. The method of claim 50, wherein the α_{1C} selective agonist activates the human α_{1C} adrenoceptor at least ten-fold more than it activates a human histamine H_1 or H_2 receptor.

55. The method of claim 50, wherein the α_{1C} selective agonist activates the human α_{1C} adrenoceptor at least ten-fold more than it activates a human dopamine D_1, D_2, D_3, or D_5 receptor.

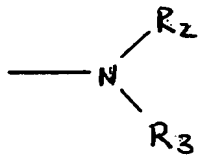
56. The method of claim 50, wherein the α_{1C} selective agonist activates the human α_{1C} adrenoceptor at least ten-fold more than it activates a human serotonin 5-HT_{1A}, 5-$HT_{1D\alpha}$, 5-$HT_{1D\beta}$, 5-HT_{1E}, 5-HT_{1F}, 5-HT_2, or 5-HT_7 receptor.

57. A method of treating urinary incontinence in a subject which comprises administering to the subject a therapeutically effective amount of a compound having the structure:



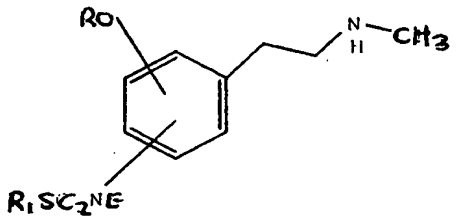
wherein n is an integer from 1 to 6; R is H or C_1-C_6 alkyl; R_1 is C_1-C_6 alkyl, phenyl, naphthyl, substituted phenyl or naphthyl wherein the

substituent is a halogen, or a C_1-C_6 alkyl or alkoxy group; wherein

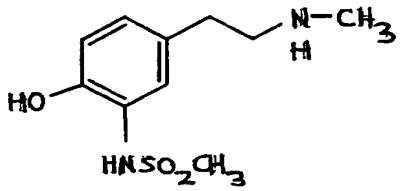


is an amino group or a heterocyclic group; the heterocyclic group is piperidine, morpholine, piperazine, pyrrolidine, hexamethylene, or thiomorpholine, the heterocyclic group being bonded through the nitrogen atom thereof to the $(CH_2)_n$ group; the amino group, wherein R_2 is H, C_1-C_6 alkyl, benzyl, or benzyhydryl and wherein R_3 is H; C_1-C_{10} alkyl; C_2-C_{10} alkenyl; C_3-C_{10} cycloalkyl or cycloalkenyl.

58. The method of claim 57, wherein the compound has the structure:

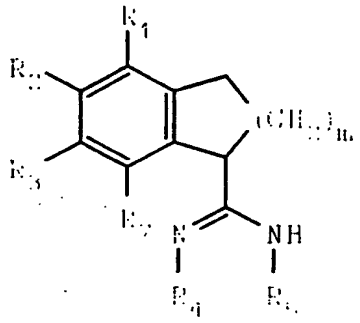


59. The method of claim 58, wherein the compound has the structure:

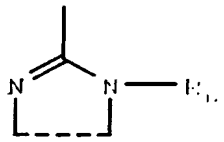


60. A method of treating urinary incontinence in a

subject which comprises administering to the subject a therapeutically effective amount of a compound having the structure:

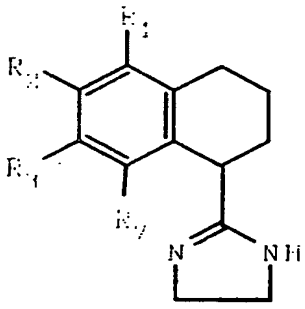


wherein m is an integer from 0 to 2; wherein each of R_1, R_2, R_3 and R_7 is independently H; OH; C_1-C_6 alkyl or alkoxy; halo; amino; acetamido or $NHSO_2R$ with R being H or C_1-C_6 alkyl; wherein R_1 and R_2 or R_2 and R_3 or R_3 and R_7 taken together constitute a methylenedioxy, ethylenedioxy, benzimidazole or indole ring; wherein each of R_4 and R_5 are independently H or taken together has the following formula:

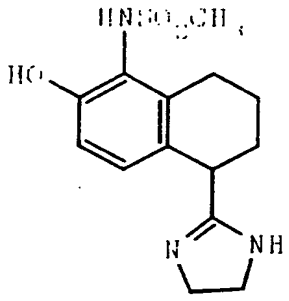


wherein the dashed line represents a single or double bond; and R_6 is H or C_1-C_6 alkyl; or a pharmaceutically acceptable salt thereof.

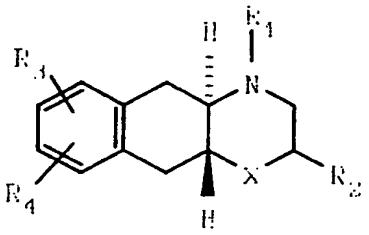
61. The method of claim 60, wherein the compound has the structure:



62. The method of claim 61, wherein the compound has the structure:

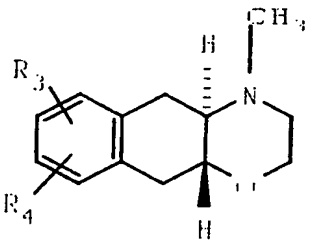


63. A method of treating urinary incontinence in a subject which comprises administering to the subject a therapeutically effective amount of a compound having the structure:



wherein each of R_1 and R_2 is independently H or C_1-C_4 alkyl; wherein R_3 is OH or C_1-C_4 alkoxy; and R_4 is C_1-C_4 alkylthio, alkylsulfoxide or alkylsulfone; Cl; Br; I or CF_3; wherein X is O, S, SO, SO_2, NH, NR_1 or $NC(O)R_1$; in free base or acid addition salt form.

64. The method of claim 63, wherein the compound has the structure:



65. The method of claim 64, wherein the compound has the structure:

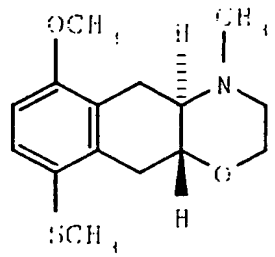


FIGURE 1A

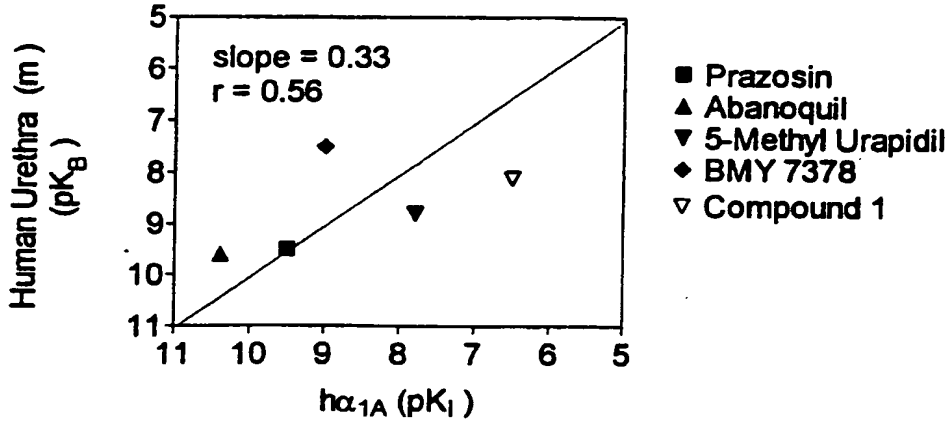


FIGURE 1B

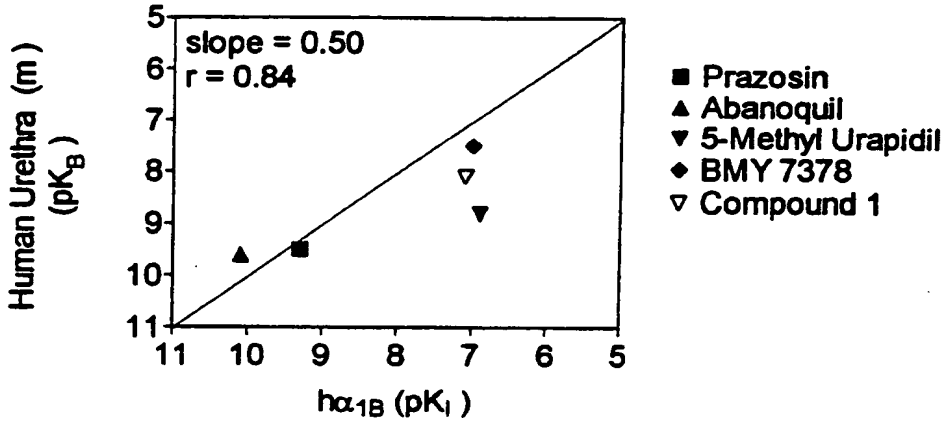


FIGURE 1C

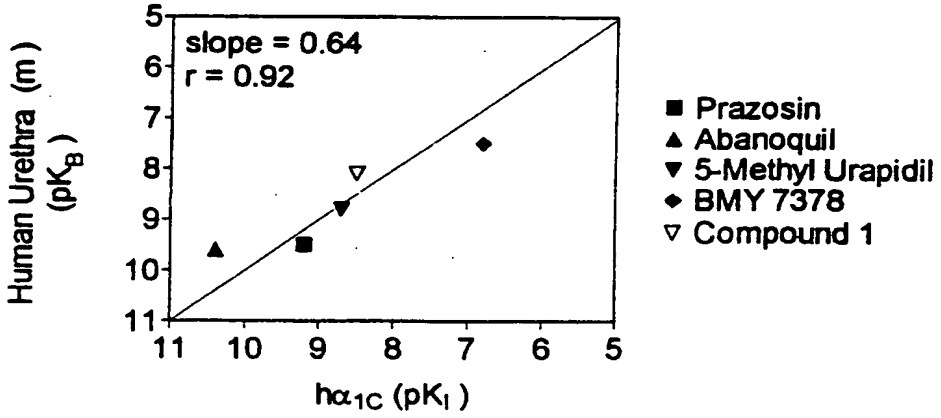


FIGURE 2A

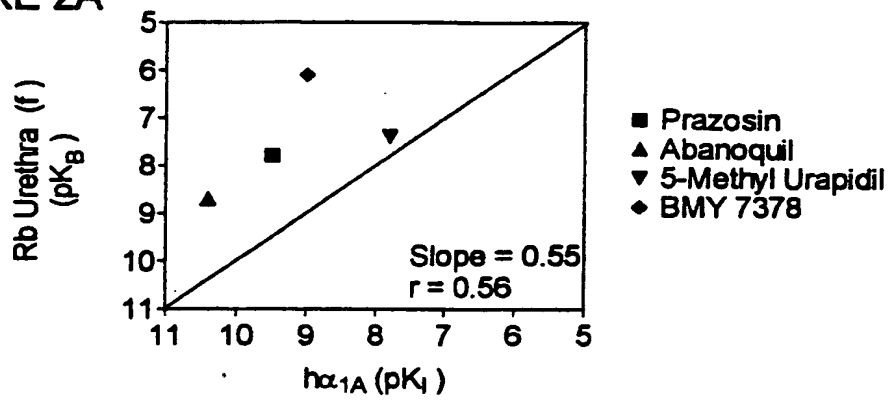


FIGURE 2B

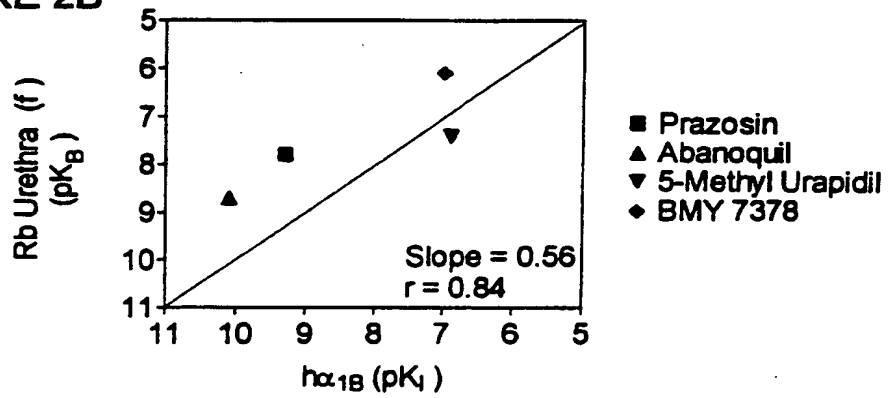


FIGURE 2C

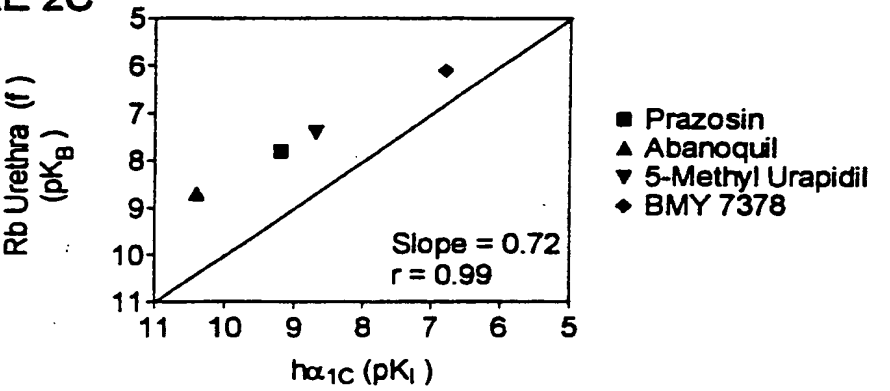


FIGURE 3A

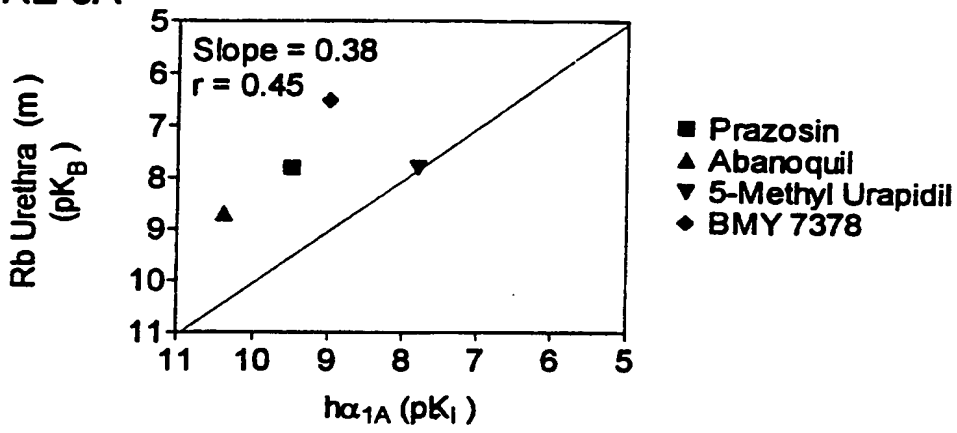


FIGURE 3B

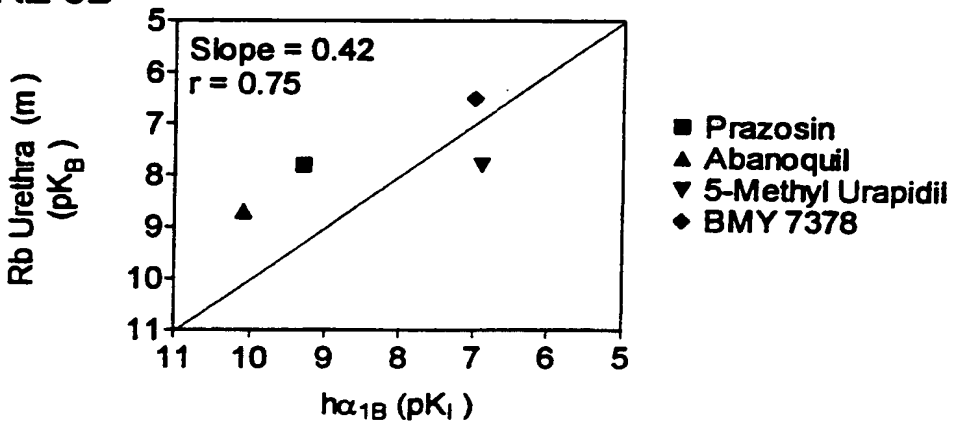


FIGURE 3C

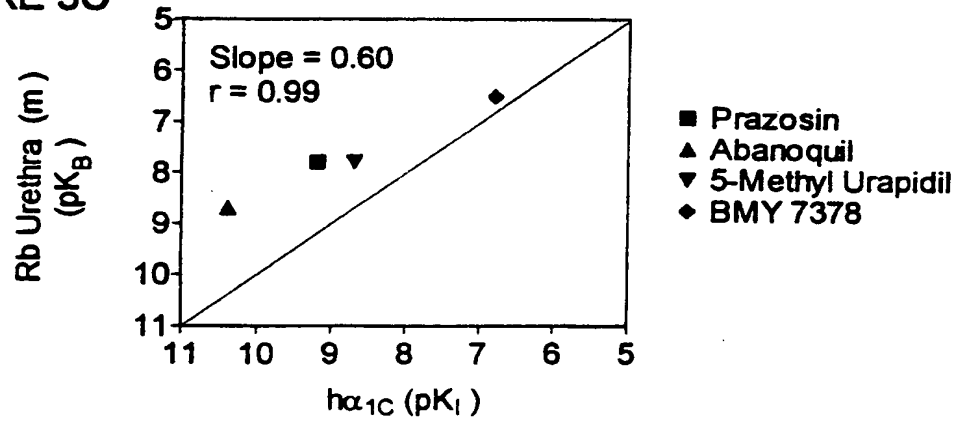


FIGURE 4A

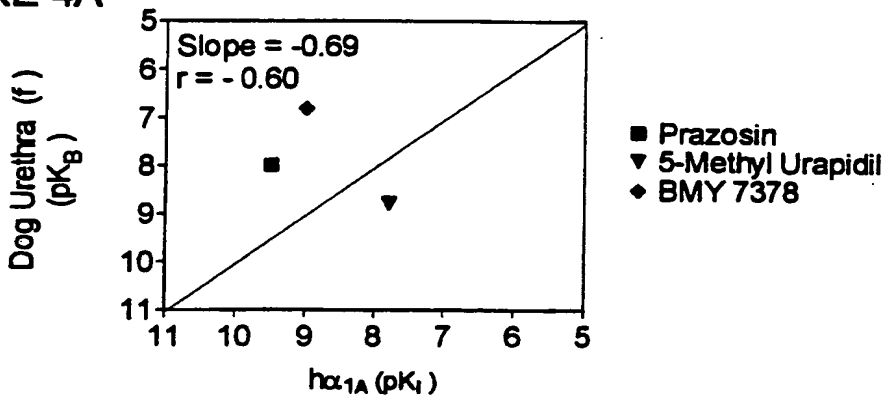


FIGURE 4B

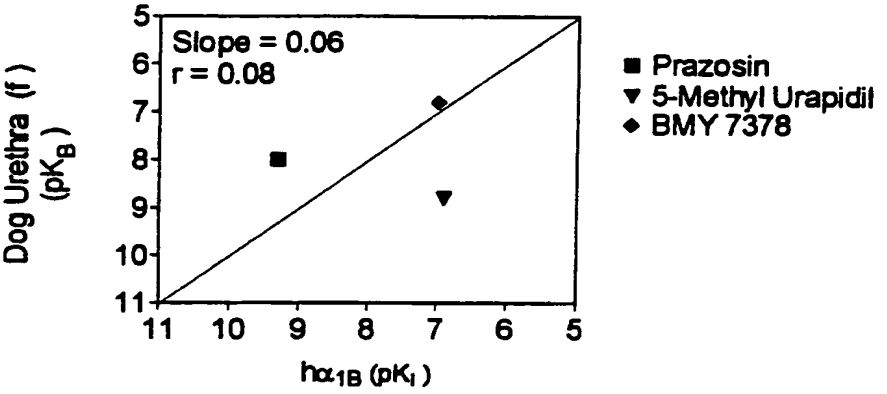


FIGURE 4C

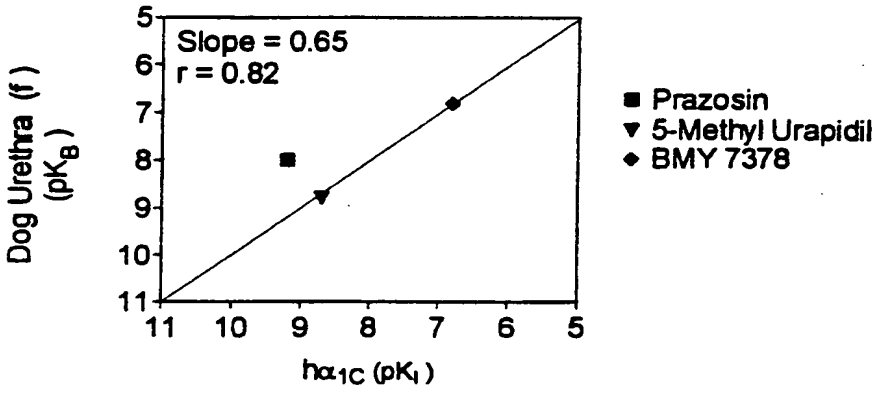


FIGURE 5A

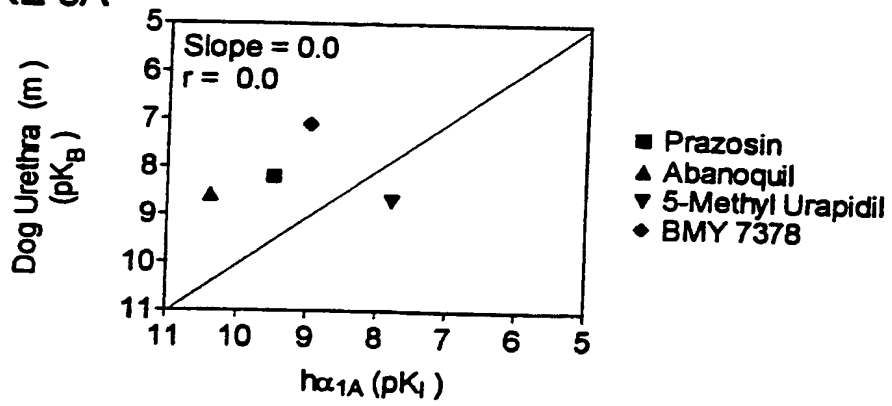


FIGURE 5B

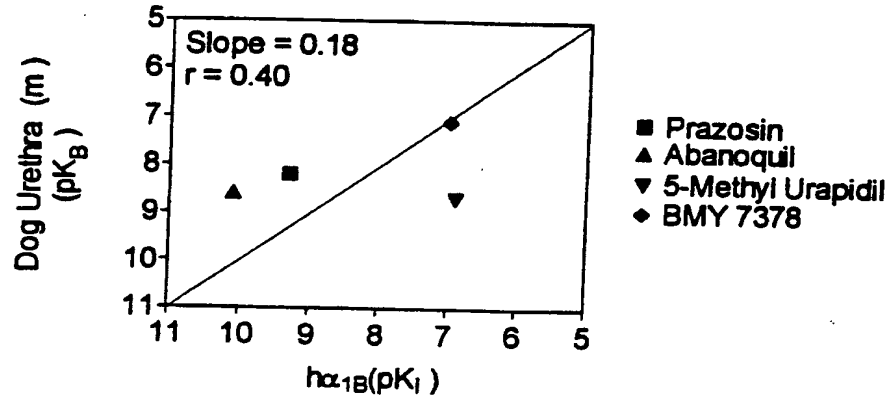


FIGURE 5C

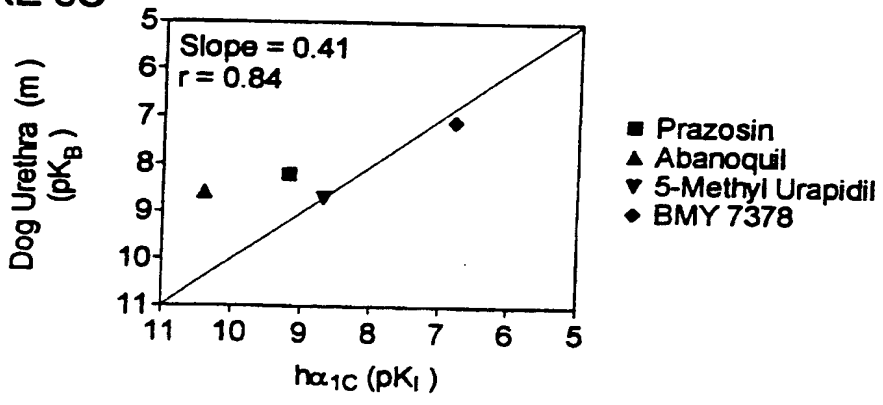
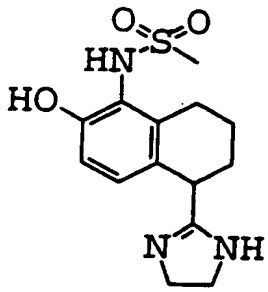
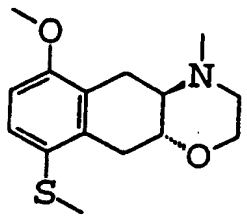


FIGURE 6

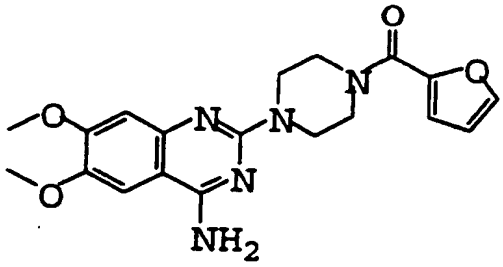
SK&F 102652



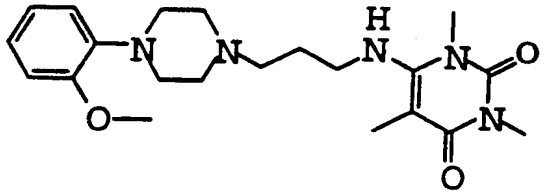
A-61603



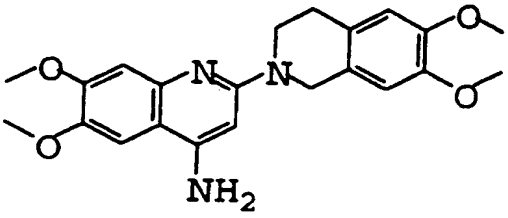
SDZ NVI 085



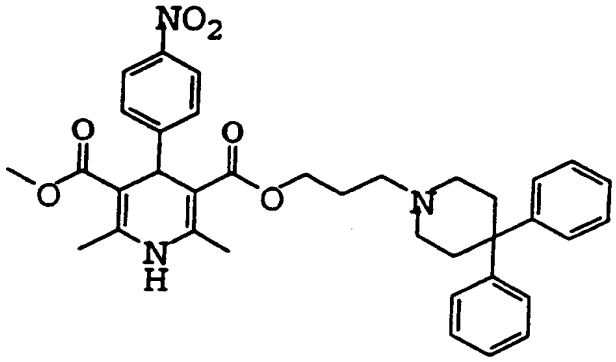
Prazosin Hydrochloride



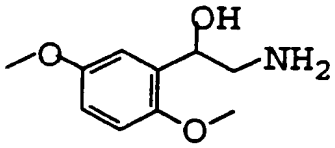
5-Methyl Urapidil



Abanoquil
Hemifumarate Hydrate



Compound 1



ST-1059

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/07979

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A61K 31/415, 31/18, 31/535
US CL :514/400, 396, 605, 402, 229.8
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/400, 396, 605, 402, 229.8

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

STN (REGISTRY (STRUCTURE), CA, BIOSIS, MEDLINE)
search terms: alpha agonist, urinary, bladder, urethra, urination, incontinence, control

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Chemical Abstracts, Volume 110, No. 17 issued 24 April 1989, Kyncl et al, "Novel adrenergic compounds. I. Receptor interactions of Abbott-54741 [(5,6-dihydroxy-1,2,3,4-tetrahydro-1-naphthyl)imidazoline] an α-adrenergic agonist" see page 70, column 2, abstract no. 110:147673v, J. Cardiovasc. Pharmacol.	1-56 & 60-62
Y	Chemical Abstracts, Volume 117, No. 1 issued 06 July 1992, Kontani et al, "Effects of adrenergic agonists on an experimental urinary incontinence model in anesthetized rabbits", see page 580, column 2, abstract No. 117:576b, Jpn. J. Pharmacol.	1-56 & 60-62

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
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Date of the actual completion of the international search	Date of mailing of the international search report
21 AUGUST 1996	13 SEP 1996
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